FY 1997 ANNUAL REPORT

OF

INTRAMURAL RESEARCH PROGRAM ACTIVITIES

OF THE

NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM

US DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
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ACRONYMS

2-DG	2-deoxyglucose
3-MC	3-methylcholanthrene
5-HIAA	5-hydroxyindoleacetic acid
$5HT_3$	serotonin type 3
ADAH	attention deficit hyperactivity disorder
ALD	alcoholic liver disease
$ALDH_2$	aldehyde dehydrogenase
ASPD	antisocial personality disorder
ATP	adenosine 5'-triphosphate
AVP	vasopressin
AWS	alcohol withdrawal syndrome
CAMT	^{11}C-α-methyltryptophan
CGSA	coarse-graining spectral analysis
CNS	central nervous system
CRH	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DHA	docosahexaenoate acid
DMPG	dimyristoylphosphatidylglycerol
DPH	diphenylhexatriene
DRG	dorsal root ganglion
DSC	differential scanning calorimetry
EFA	essential fatty acids
ERP	event-related brain electrical potentials
Et	ethanol
FAS	fetal alcohol syndrome
FDA	Food and Drug Administration
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
$GABA_A$	γ-aminobutyric acid type A
GS/MS	gas chromatography/mass spectrometry
HCV	hepatitis C virus
HNE	4-hydroxynonenal
HPA	hypothalamic-pituitary-adrenal
HRV	heart rate variability
HSP47	heat shock protein 47
HVA	homovanillic acid
IBI	inter-beat interval

LC	liquid crystalline
LC/MS	liquid chromatography/mass spectrometry
LS	long-sleep
LVA	low voltage alpha
MAD	mass allele detection
MAS	magic angle spinning
m-CPP	m-chlorophenylpiperazine
MDA	malondialdehyde
MHPG	3-methoxy-4-hydroxyphenyl glycol
MII	metarhodopsin II
MPG	major pelvic ganglion
MRI	magnetic resonance imaging
nACh	nicotinic acetylcholine
NER	nucleotide excision repair
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
PC	phosphatidylcholines
PE	phosphatidylethanolamines
PEG	polyethylene glycol
PET	positron emission tomography
PKA	protein kinase A
PKC	protein kinase C
PNS	parasympathetic nervous system
PS	phosphatidylserine
PTSD	post traumatic stress disorder
SAD	seasonal affective disorder
SGA	small for gestational age
SNS	sympathetic nervous system
SSCP	single-strand conformational polymorphism
TCA	tricarboxcyclic acid
TDO_2	tryptophan 2,3-dioxygenase
TDT	transmission/disequilibrium test
TPQ	Tridimensional Personality Questionnaire
TPH	tryptophan hydroxylase
TTX	threshold tetrodotoxin

FY 1997 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1996 - 30 SEPTEMBER 1997)

LABORATORY OF CLINICAL STUDIES

MARKKU LINNOILA, M.D., Ph.D., ACTING CHIEF

DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
NATIONAL INSTITUTES OF HEALTH

SYNOPSIS

LABORATORY OF CLINICAL STUDIES

INTRODUCTION

During FY 1997, the 11-bed ward of the laboratory continued to be one of the busiest inpatient services in the Clinical Center. Long-term, high risk projects were expanded in the area of brain imaging, to develop new serotonin receptor and turnover quantifying ligands. The unique brain imaging resources and expertise at the NIH Clinical Center make these efforts particularly important and potentially rewarding. These projects are, however, characterized by high risk because the metabolism and receptor binding characteristics of many of the candidate ligands have not been extensively investigated in humans.

SECTION OF BRAIN ELECTROPHYSIOLOGY AND IMAGING

Investigators in the Section of Brain Electrophysiology and Imaging conduct sophisticated electrophysiological, neuropsychological and brain imaging studies on alcoholics, individuals at risk and carefully matched controls.

The lack of an acceptable method for determining statistical significance of differences in brain images derived from functional magnetic resonance imaging (fMRI) or positron emission tomography (PET) studies has been a major problem for researchers in these areas. Over the past several years, we have made significant progress in applying rigorous statistical methods, based on a Gaussian random field model, to the analysis of image data. In addition, work on other methods for determining the statistical significance of differences observed in arbitrary regions of group average images are being developed. These include methods of spatial frequency decomposition as well as wavelet analysis. We have compared the relative merits and shortcomings of these methods with Gaussian random field based techniques. Gaussian random field based techniques are the most conservative statistically and give the most precise spatial localization; they are particularly suited to analysis of FDG PET data and we are currently examining their utility for blood flow PRT scans. Wavelet based methods do not yield as precise a spatial definition of regions of significant difference but they appear to be particularly useful in the analysis of fMRI data.

Using all three image analysis techniques, we have been able to demonstrate significant differences in glucose metabolism in the brains of normal controls and individuals who have developed anti-social behavior following a serious closed head injury. Analysis was performed using both absolute pixel glucose uptake values and means-adjusted pixel values obtained by subtracting the overall mean brain glucose uptake from each pixel glucose uptake. Both mean-adjusted and absolute glucose uptake produced similar results. Head injured subjects had significantly lower glucose uptake in the posterior orbital cortex bilaterally, in the right caudate, in the right dorsal thalamus and in the right mesial superior frontal cortex. Glucose uptake in the caudate, thalamus and superior frontal cortex were significantly correlated with ratings of aggressive behavior developed after the injury. These results suggest that a basal ganglia thalamocortical circuit, involving the orbital and mesial frontal cortex and located primarily in the right hemisphere, is important in the control of aggression and social behavior.

Work on the development of advanced image analysis and coregistration for PET, CT, structural and fMRI has continued. Methods to achieve the 3-D registration of PET images with structural MRI have been developed as have techniques for the automated detection of midsagittal lines or planes. Using these techniques, we can now identify structures as small as the head of the caudate nucleus or nucleus accumbens on coregistered PET and MRI scans.

Fully automated segmentation techniques have been developed for use on T-1 weighted MRI images. These techniques allow for the automated labeling of cerebrospinal fluid (CSF), white and gray matter regions in structural MRI data. We have applied these techniques and found evidence for selective reduction in the volume of the hippocampus among alcoholics. In addition, we have found both gender and laterality differences in this structure, among both normals and alcoholics.

We have developed a series of oculomotor tasks that can be used to assess inhibitory function, by measuring the ability to maintain visual fixation in the presence of distracters. We used four oculomotor tasks to measure the inhibition of contextually inappropriate saccades in alcoholic inpatients and control subjects. Two smooth pursuit tasks were used to evaluate ability to suppress saccades during visual pursuit tracking. During the two smooth pursuit tasks, the alcoholics performed as well or slightly better than the control subjects. We also used two oculomotor tasks designed to elicit context appropriate saccades and require suppression of context inappropriate saccades. The first of these was a stepping task in which the target stepped, at random intervals, between two fixed locations. This task required subjects to inhibit saccades elicited by an internal representation of the target's expected behavior while making saccades when the target actually stepped to the new location. During this task, alcoholic patients had a shorter latency (oculomotor reaction time) than control subjects for context-appropriate visually-guided saccades, suggesting a failure to maintain attentional fixation in the presence of anticipated target behavior. The second was a go/no-go task during which subjects had to make saccades to targets while ignoring visually-salient non-targets. During this task, the alcoholic patients made more saccadic errors of commission (saccades directed at non-targets) than the control subjects. These results demonstrate that, among our sample of alcoholics, there was no impairment of smooth pursuit eye movement or failure to inhibit saccades during visual pursuit tracking. However, when the alcoholics had to maintain attentional fixation in the face of internal or external distracters, they failed more often than control subjects. Failure to inhibit inappropriate saccades may be associated with other types of inhibitory deficits observed among alcoholics.

SECTION OF CLINICAL ASSESSMENT AND BIOLOGICAL CORRELATES

The Section of Clinical Assessment and Biological Correlates clinically evaluates subjects for genetic, physiological and biochemical studies conducted by other investigators in the Laboratory and the Division, as well as for studies conducted within the Section. Research currently in progress includes studies comparing different subgroups of alcoholics, both men and women, in order to elucidate risk factors for alcoholism and impulsive behaviors such as suicide attempts, physical violence and drug abuse. There is an additional focus on clinical personality traits within these subgroups. The Section is also in the process of assessing psychiatric diagnoses and characteristics of alcoholism in American Indians, African-Americans and Caucasians (Americans and Finns); collaborations with other groups are predominant.

Developmental studies involving aggressive, impulsive and conduct-disordered children are underway, both within the Laboratory and in collaboration with the Laboratory of Developmental Psychology, NIMH.

This Section has initiated the use of computer-assisted programs in diagnostic assessments.

SECTION OF CLINICAL SCIENCE

The major objectives of research conducted in the Section of Clinical Science are to: (1) characterize the role of various neurotransmitter systems in the etiology of alcoholism by utilizing CFS metabolite determinations, pharmacological challenge paradigms and PET studies; (2) explore possible biochemical determinants that might differentiate subtypes of alcoholics; (3) describe and understand the behavioral and biochemical interactions among patients with alcoholism, panic disorder and violence; (4) explore gender differences, among patients with alcoholism, using a serotonin challenge paradigm; (5) investigate the effect of

glucoprivic stress on hypothalamic function in patients with alcoholism; (6) use procaine as a probe for limbic system function in alcoholics with and without panic disorder; (7) introduce new pharmacological interventions for long-term treatment of alcoholism; (8) study the psychological and biological effects of smoking cessation in detoxifying alcoholics; (9) characterize the concept of "losing control" as it relates to violent behavior and alcoholic drinking; (10) describe changes accompanying protracted withdrawal from alcohol in brain neurotransmitter and neuropeptide hypothalamic-pituitary-adrenal (HPA) axis functioning and magnesium and zinc concentrations; and, (11) study the effects of hepatitis C virus (HCV) infection in alcoholics.

Administration of the serotonergic ligand, m-chlorophenylpiperazine (m-CPP), to detoxified male alcoholics resulted in different responses between early-onset (type II) and late-onset alcoholics (type I), with early-onset alcoholics more likely to report a "craving" for alcohol, while late-onset alcoholics reported more anger and anxiety. Possible differences in serotonergic functions between subtypes of alcoholics were substantiated by CSF analyses. Abstinent alcoholics, who had the onset of alcoholism before the age of 25, had lower 5-HIAA concentrations compared to those who had the onset of alcoholism after the age of 25. Results from other challenge paradigms suggest that there may be other biological differences between alcoholics and controls. Administration of the physiological stressor, 2-deoxyglucose (2-DG), to three-week abstinent alcoholics resulted in an exaggerated ACTH response compared to controls. Both, baseline insulin and insulin release (AUC) following 2-DG, showed a significant positive correlation with the quantity of alcohol consumed per occasion during the previous six months. Administration of sodium lactate, to detoxified alcoholics with panic disorder, resulted in a lower frequency of panic attacks compared to nonalcoholic panic disorder subjects. This difference was not explained according to whether the panic disorder started before or after the onset of alcoholism. Administration of dextrose in lactate decreased the likelihood of panic subjects experiencing a lactate-induced panic attack. Intravenous administration of the local anesthetic, procaine, resulted in an increased frequency of panic attacks in panic patients (with and without alcoholism) compared to alcoholics and controls.

Preliminary results indicate that 70% of alcoholics randomized to stop smoking were not able to remain smoke-free for the month-long study. Analysis of CSF monoamine metabolite showed nonsmoking alcoholics had significantly increased concentrations of HVA compared to smoking alcoholics. There were no group differences in CSF 3-methoxy-4-hydroxyphenyl glycol (MHPG) and 5-HIAA.

Individuals who "lose control" and become physically violent have a higher than expected prevalence of alcoholism, panic disorder, borderline personality and obsessive-compulsive personality disorder. Clinically, patients described a number of physical symptoms (palpitations, shortness of breath, shaking, sweating) as well as cognitive symptoms (losing control, depersonalization, fear) prior to "going off" and becoming violent. Mid-term analysis of the data reveals that perpetrators of domestic violence are different from nonviolent alcoholics and controls as follows: 1) perpetrators are significantly more likely to experience either panic and/or rage during a lactate infusion; 2) perpetrators have lower CSF concentrations of the serotonin metabolite, 5-HIAA, than nonviolent alcoholics; 3) perpetrators demonstrate an inability to regulate their autonomic nervous system as evidenced by a decreased relationship between vagal tone and heart rate; and, 4) violent alcoholics, compared to nonviolent alcoholics, show a decrease in glucose utilization in the orbital frontal brain region.

We have found no major histological differences in severity of liver disease among HCV(+) and HCV(-) alcoholics and HCV(+) nonalcoholic blood donors.

Preliminary results suggest that serum ionized magnesium does not reflect tissue ionized magnesium and that ionized magnesium is regulated in a tissue-specific manner.

Detoxified alcoholics demonstrate a specific type of cognitive impairment which is expressed as a failure to inhibit errors in learning, remembering and attention and an associated impairment in ability to track the source of remembered knowledge. This is evident under unstructured information processing conditions but not when they are directly asked to monitor and evaluate their own cognitive performance. Polydrug abusers demonstrate a similar type of cognitive deficit. The cognitive style of alcoholics and polydrug abusers are more likely to be defined by external stimuli rather than conceptually (internally) driven cognitive processing that characterizes the information processing style of normal volunteers. The performance of alcoholics on tasks that require reflective functions, such as explicit remembering and inhibition of intrusions, is not correlated with cerebrospinal fluid (CSF) measures of the neurotransmitter metabolites, 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA), nor neuroanatomical variables as assessed by brain imaging methods. We are currently exploring whether the "cognitive style" used by alcoholics is a risk factor for development of alcoholism. Ongoing studies of children at risk for alcoholism as well as genetic-twin studies of alcoholics will allow us to test this hypothesis.

The cognitive changes associated with normal aging (and depression) are qualitatively different from the cognitive impairments apparent in detoxified alcoholics. Large, highly reliable and generally linear, aging-related declines in cognitive functioning are apparent for all of the cognitive domains cited above and, no single proposed model of cognitive aging accounts for the broad and robust changes in cognitive functioning associated with aging. The pattern of aging-related changes in cognition is discriminable from those expressed in detoxified alcoholics as well as those in early Alzheimer's disease.

Benzodiazepines potentiate preexisting impairments in reflective (control) cognitive operations in detoxified alcoholics and polydrug abusing patients. This is expressed as a selective impairment in ability to suppress intrusions, monitor the source of remembered knowledge, attend to information without the benefit of orienting cues, encoding functions under unstructured processing conditions and the perception of ambiguous information. The effects of benzodiazepines on attention are similar to those produced by alcohol and are the opposite of attentional effects associated with aging. Furthermore, unlike normal volunteers, detoxified alcoholics demonstrate a robust qualitative shift in how they think about standard stimuli.

The effect of alcohol on cognitive functions is similar to that observed following the administration of a benzodiazepine and different from the response to other classes of drugs, i.e., cholinergic antagonists. Effects of other benzodiazepines, such as alprazolam and adinazolam, produce similar dose-dependent sedative and cognitive effects which simulate the cognitive deficits expressed in untreated amnestic patients. The selective cognitive impairing effects of benzodiazepines are, at least, partially independent of their sedative effects. The effect of the anesthetic, ketamine, an antagonist of the N-methyl-D-aspartate (NMDA)-type glutamate receptor (thought to be involved in memory consolidation) mimics the cognitive changes expressed in normal aging. This receptor has been shown to be altered by alcohol. Paradoxically, subjects that acquire knowledge prior to the administration of a benzodiazepine, such as triazolam, demonstrate a facilitation in recall of that information (compared to recall tested under placebo conditions).

The profile of highly specific effects of benzodiazepine (and alcohol) on reflective functions is thought to be important in understanding patterns of uncontrolled drinking and may provide information on the stimulus-discriminative (and reinforcing) properties of these drugs in terms of the selective aspects of autobiographical memory that are elicited under drug in contrast to undrugged conditions. The specific types of cognitive changes that are induced by benzodiazepines (and alcohol) are currently being developed to provide models of the cognitive changes apparent not only in the amnestic alcoholic, but also in nominally unimpaired, detoxified alcoholics and knowledge that can be used to more effectively treat patients with alcoholism.

SECTION ON NEUROCHEMISTRY AND NEUROENDOCRINOLOGY

The Section on Neurochemistry and Neuroendocrinology has continued research on biochemical concomitants of violent behavior in alcoholics and on variables associated with increased vulnerability of developing alcoholism-related behavior. The major focus of this research has remained the serotonergic system. Interesting insights have been gained into regulation of serotonergic neuronal networks, developmental and genetic influences on serotonin functions and serotonergic regulation of energy metabolism and excessive alcohol consumption.

UNIT OF PHARMACOKINETIC STUDIES

Research efforts of the Unit of Pharmacokinetic Studies primarily focused on the development of appropriate kinetic models to describe and quantify the movement of various tracers to be utilized in PET studies. Alpha-methyl-L-tryptophan continues to be investigated as a tool to determine if there are differences in tryptophan uptake and serotonin synthesis rates in various brain regions in non-human primates and humans.

Research concerning the determination of the kinetics of new therapeutic agents, that are being evaluated as treatments in non-human primates, for anxiety-mediated and/or stress and non-stress conditions is also being pursued.

Another area of investigation is the role of alcohol dehydrogenase genotype and glucose metabolism in ethanol elimination. Ethanol elimination rates will be compared in individuals with different ethnic backgrounds (European Caucasian, African-American, Hispanic and Asian) but similar alcohol dehydrogenase genotypes. Men and women will be given doses of ethanol, based on total body water, to account for differences in ethanol distribution between the sexes and ethnic groups.

The role of a second enzyme pathway, in the elimination of ethanol, is being investigated by developing a two-compartment open pharmacokinetic model that incorporates dual Michaelis-Menten based elimination pathways. Ethanol will be given to both men and women intravenously, to avoid absorption differences, and doses will be given as g/l total body water to account for differences in ethanol distribution.

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INTRAMURAL RESEARCH PROJECT ZO1 AA 00279-08 LCS
October 1, 1996 to September 30, 1997

Title of Project: Psychopathology in African American Alcoholics

Principal Investigator: V. Moore, M.S.W. (Research Assistant)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: I. Culver, LCS
M. Linnoila, M.D., Ph.D., LCS

Collaborating Units: None

Staff-Years: 0.25

Sample Type: Human subjects (Minors & Interviews)

Summary of Work: Data have been collected and analyzed, a manuscript has been prepared and submitted and is undergoing consideration for publication. This project is now terminated.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00002-05 LCS
October 1, 1996 to September 30, 1997

Title of Project: Eye Movement in Alcoholism and Individual at Risk for Alcoholism

Principal Investigator: D.W. Hommer, M.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: M. Israel, LCS

Collaborating Units: Child Psychiatry Branch, NIMH (J. Rapoport, M.D.); Seattle VAMC (A. Radant, M.D.)

Staff-Years: 0.4

Sample Type: Human subjects

Summary of Work: The study of human eye movements provides an extremely useful approach to the examination of a variety of cognitive functions. It is obvious that the latency and goal saccadic eye movements are related to attention. What is not so obvious is that other aspects of cognition such as short-term memory, preparatory set, and inhibition of context inappropriate responses can also be assessed using eye movement techniques. Short-term memory, preparatory set, and inhibition of context inappropriate responses constitute core functions of the prefrontal cortex, the brain region most involved in the control of higher order cognitive processes. We have used a number of different tasks to elicit saccades, including Go/No-Go tasks and delayed response tasks. These tasks allow us to independently assess core functions of the prefrontal cortex by measuring the accuracy and latency of memory guided saccades, as well as the frequency of context inappropriate saccades that should be inhibited. Using these tasks we have demonstrated that schizophrenics are impaired in all three core aspects of prefrontal cortex function while children with attention deficit hyperactivity disorder (ADHD) are impaired in only their ability to inhibit context inappropriate saccades. During the past year we have demonstrated that, similar to children with ADHD, alcoholics are impaired in their ability to inhibit context inappropriate saccades. The smooth pursuit eye movements of alcoholics are completely normal. We are in the process of extending our eye movement recording equipment so that we will be able to measure vertical as well as horizontal eye movements. This addition will allow us to develop new tasks examining modulation and control of visual attention.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00061-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Cerebral Metabolic Correlates of Aggressive and Addictive Behavior

Principal Investigator: D.W. Hommer, M.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.T. George, M.D., LCS
M. Linnoila, M.D., Ph.D., LCS
D.E. Rio, Ph.D., LCS
U.E. Ruttimann, Ph.D., LCS

Collaborating Units: Nuclear Medicine Department, CC, NIH (P. Herscovitch, M.D.)

Staff-Years: 2.5

Sample Type: Human subjects

Summary of Work: This research is designed to determine neuroanatomical and neurochemical correlates of addictive and aggressive/impulsive behavior in human subjects. The principal focus of these studies is the measurement and correlation of regional cerebral glucose metabolic activity using PET, brain volumes using MRI, CSF metabolites and measures of impulsive/aggressive behavior and excessive alcohol consumption. During the past year, we have completed a PET study comparing serious head injury and secondary sociopathy. Examining the brains of head injured persons with secondary sociopathy provides insight to structural and functional pathology associated with antisocial behavior and may also provide insight to sociopathy not acquired as a result of head trauma. When we compared the energy use in the brains of twelve healthy men with ten men who became irresponsible, socially inappropriate and impulsively aggressive following a serious head injury, we found significantly lower energy use in five brain regions only -- the lateral orbital cortex on both sides of the brain, a region of the right prefrontal cortex known as Brodman area 32, the right medial thalamus and the right caudate nucleus. Energy use of the entire brain was not lower among the head injured men. The largest differences in brain energy use between normal and head injured men were in the right medial prefrontal cortex, the right thalamus and right caudate. Energy use in these three brain regions was closely associated with the severity and amount of aggressive behavior in which subjects had engaged since their injury. Comparison of the size of brain structures using MRI yielded results similar to our PET results. The volume of the entire brain did not differ between the two groups, but the volumes of the right medial prefrontal cortex (Brodman area 32), the right thalamus and right caudate were significantly smaller among the head injured men compared to the healthy men. The size of these three regions on the left side of the brain did not differ between the head injured and healthy men. We have also continued studies of brain structure in alcoholics and controls. Controlling overall brain volume, we have found significant reductions in the volumes of the hippocampus in alcoholics. Studies of other brain regions such as thalamus, caudate and mesial frontal cortex are planned.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00062-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Brain Serotonin Synthesis in Patients with Addictive and Aggressive Behaviors

Principal Investigator: D.W. Hommer, M.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.T. George, M.D., LCS
M. Linnoila, M.D., Ph.D., LCS
D.E. Rio, Ph.D., LCS
S.E. Shoaf, Ph.D., LCS

Collaborating Units: Nuclear Medicine Department, CC, NIH (W. Eckelman, M.D.; B. Schmall, Ph.D.)

Staff-Years: 2

Sample Type: Neither human subjects nor tissues

Summary of Work: This project was designed to develop and implement a novel positron emitting tracer ^{11}C-α-methyltryptophan (CAMT) in order to measure the local distribution and accumulation of brain serotonin in patients with alcoholism, varying degrees of aggressive/impulsive behavior and in normal volunteers.

CAMT is currently not approved for human use in the United States. In order to develop CAMT for humans, we must provide toxicology and dosimetry data that fulfill Food and Drug Administration (FDA) and Radiation Safety Committee guidelines, gather kinetic data in higher animals and modify the synthesis to produce the L-form of CAMT.

The preclinical toxicology in rabbits and mice was completed. The dosimetry studies in rhesus macaques, to measure individual organ exposure to CMAT, had also been completed. The synthesis of L-CAMT was running well and sufficient quantities of L-CAMT could be produced.

Preliminary to using L-CAMT in humans, we had been performing L-CAMT PET scans on 12 rhesus monkeys whose social behavior had been observed and rated since birth. In addition, multiple CSF samples were analyzed for serotonin metabolites (5-HIAA) in all study animals. Study results show that the L-CAMT method for determining serotonin synthesis rates had several serious methodological and theoretical shortcomings which preclude its use in humans; therefore, this project is being terminated.

See report ZO1 AA 00098-01 LCS for details.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00063-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: EEG Studies of Electromotive Generators Affected by Alcohol

Principal Investigator: D.W. Hommer, M.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: C. Adams, Ph.D., LCS
A. Westdorp, Ph.D., LCS
S. Law, LCS

Collaborating Units: Washington University, Dept Psychiatry (J. Rohrbaugh, Ph.D.); Tulane University (P. Nunez, Ph.D.); Rensselaer Polytech Institute (J. Goble, Ph.D.); INSERM CJF 90-12 (Rennes, France)/Brain Institute, UCLA (E. Halgren, Ph.D.); National Center for Research Resources, NIH (B. Roth, Ph.D.)

Staff-Years: 2

Sample Type: Human subjects

Summary of Work: The long-term objective of this research is the quantification of acute and chronic effects of alcohol on electromotive generators in the brain and the determination of relationships of abnormal generators and predisposition to excessive alcohol consumption. Research integrates the development of high-resolution EEG methods to localize and study electromotive generators of brain processes with various electrophysiological models, both computational and theoretical, to validate the methods and test hypotheses of suspected generators. This integrated procedure is used to determine how, when, and where alcohol alters mechanisms which process sensory input, respond to input, and store and retrieve information. Sensory input is controlled in three primary sensory modalities: visual, auditory, tactile. In addition to investigating the processing in these individual sensory systems, the processing in additional brain systems that mediate the interaction between these sensory systems are being investigated. Event-related brain electrical potentials (ERPs), which provide both spatial and temporal information about these mechanisms, and nonstationary EEGs, which provide a measure of the brain's dynamic interactions, are used in this investigation. A physical, artificial head model is also used to study the nonhomogeneous and anisotropic nature of volume conduction in the head. Methods have been developed that improve the spatial resolution of current EEG methods by a factor of three.

Preliminary findings suggest that temporal resolution is improved as well. These methods have been shown to provide better estimates of underlying brain processes than present techniques and are more insensitive to electrophysiological artifacts such as eye blinks.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00064-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Analysis of Brain Images

Principal Investigator: D.E. Rio, Ph.D. (Staff Scientist)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.W. Hommer, M.D., LCS
R. Rawlings, LCS
U.E. Ruttimann, Ph.D., LCS
W. Williams, M.D., LCS

Collaborating Units: MedData, McLean VA (R. Momenan, Ph.D.)

Staff-Years: 4

Sample Type: Human subjects

Summary of Work: Traditional methods to analyze image data from PET, MRI and fMRI have proven only partially successful. This is due, in part, to the inherent biological variability, physical limitations of the acquisition instrumentation and mathematical algorithms applied to reconstruct the image data, but it also reflects the inadequacy of the computational, mathematical and statistical methods employed in analysis of these data. Image data acquired by PET and MRI have numerous sources of distortion. Depending on the imaging modality, these appear as spatial distortions, decreases in signal-to-noise ratio, modification of image values and increased spatial correlation. PET and MRI data can be "improved" by using appropriate models to restore and analyze the reconstructed image. Methods in both the spatial domain, using the theory of Gaussian random fields and Fourier, or frequency domain have been developed for analysis of PET and fMRI. In order to evaluate these models, simulated PET brain intensity data and PET and MRI brain shape data have been created using empirically measured image characteristics. In particular, Monte Carlo techniques have been developed to create groups of PET data with known attributes and specific group differences. The control of signal and noise associated with these models allows us to evaluate the effect of geometric distortions and sensitivity of identification of localized statistically significant differences between the groups. In the case of geometric models, it is possible to create 3-D brain (or skull) shapes with known noise to evaluate the limitations of rescaling PET images across subjects to a given standard and registration for the same subject. These simulations are being used to study two related areas currently under investigation: (1) statistical techniques are being researched for both the geometric and grey scale values of PET and MRI data; and (2) the precision of multimodality 3-D superposition of functional and structural images obtained for PET and MRI data, especially when the data are sparse or have known symmetries.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00065-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Semi-Automated Methods of Segmentation of Brain Images

Principal Investigator: U.E. Ruttimann, Ph.D. (Staff Scientist)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.W. Hommer, M.D., LCS
D.E. Rio, Ph.D., LCS

Collaborating Units: MedData, McLean VA (R. Momenan, Ph.D.); National Center for Research Resources, NIH (P. Thevenaz, Ph.D.; M. Unser, Ph.D.)

Staff-Years: 2

Sample Type: Human subjects (Interviews)

Summary of Work: The establishment of associations between structure and function among various areas of the brain is an important step in the identification of neurologic mechanisms in both normal and disease states. The pursuit of this goal requires the geometrical coregistration of digital images in two types of major applications: (1) data fusion, in case brain images from the same subject were acquired by different modalities; (2) data comparison, for the detection of significant differences between different subject groups in images acquired by the same modality. The first application requires identification and delineation ("segmentation") of distinct areas and landmarks in the brain. A procedure based on dynamic clustering and region growing algorithms has been developed that segments T1-weighted MR images into regions of CSF, gray and white brain matter. Its application to MR images of alcoholic and normal subjects yielded results consistent with a subjective segmentation. Automated methods for excluding extracranial tissues still need to be developed.

For the second (within-modality) application, the gray-level information, itself, can be employed for image registration without the need for segmentation. A multi-scale registration procedure has been developed that determines parameters of a general 3-D affine transformation (translation, rotation about an arbitrary center, anisotropic scaling and skewing) between volumes to be registered that minimizes the average squared gray-level difference between corresponding voxels. Successful registration of PET images achieving homogeneous registration variance across the entire brain section has been achieved for both within and between subject analyses. The method is also used for the coregistration of long time series of volumes acquired in fMRI studies. An extension of this method is being developed based on using the cross-entropy between different volumes as a similarity measure instead of the gray-level difference; with this extension, cross-modality registration is feasible. Automated coregistration of MR images (T1) to PET images of the same subject has been achieved and performance comparisons relative to landmark-based registration is in progress.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00081-04 LCS
October 1, 1996 to September 30, 1997

Title of Project: Functional Magnetic Resonance Imaging of Olfactory Stimulus Processing

Principal Investigator: D.W. Hommer, M.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: R. Rawlings, LCS
D.E. Rio, Ph.D., LCS
U.E. Ruttimann, Ph.D., LCS

Collaborating Units: National Center for Research Resources, NIH (P. Van Gelderen, Ph.D.; C. Moonen, Ph.D.); LDRR (S. Duyn, M.D.)

Staff-Years: 2

Sample Type: Human subjects

Summary of Work: Unlike visual or tactile perception, the functional anatomy of odor perception in humans has received very limited attention. This is unfortunate because the brain regions involved in odor perception appear to overlap with the brain regions involved in motivation and emotion. Since, in alcoholics, states of craving for alcohol can be induced by the odor of alcoholic beverages and these states involve both motivational and emotional components, we felt, as a prelude to studies of the functional neuroanatomy of alcohol craving, it would be important to develop techniques to examine brain changes associated with olfactory perception.

Normal volunteers were exposed to various odorants using a continuous airflow system, while lying in a standard 1.5 Tesla MRI scanner. A pulse sequence developed at the *In Vivo* NMR Center was used to image blood volume under controlled conditions. Most foci of signal intensity change were located in secondary olfactory areas, such as amygdala, entorhinal cortex, nucleus accumbens/septal nuclei and some in orbital frontal cortex. Different sites of changes were found in different subjects, possibly due to the relatively low sensitivity of this novel brain imaging method. However, only 25% of normal subjects show significant changes in signal intensity. For this reason it was felt that fMRI, at present, does not have sufficient power to image brain response to odors. ^{15}O PET appears to hold greater promise for imaging effects of olfactory stimulation, in order to compare the distribution of changes in cerebral blood flow measured during PET, in both alcoholics and controls. Controls demonstrate a significant increase in cerebral blood flow during exposure to the odor of alcohol containing beverages in primary and secondary olfactory cortex. In contrast, alcoholics show less robust activation in primary and secondary olfactory cortex during exposure to the odor of alcohol containing beverages. These results are consistent with hyporesponsivity of limbic brain regions among alcoholics.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00082-04 LCS
October 1, 1996 to September 30, 1997

Title of Project: Statistical Analysis of Image Features

Principal Investigator: U.E. Ruttimann, Ph.D. (Staff Scientist)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.W. Hommer, M.D., LCS
R. Rawlings, LCS
D.E. Rio, Ph.D., LCS

Collaborating Units: MedData, McLean, VA (R. Momenan, Ph.D.; National Center for Research Resources, NIH (M.Unser, Ph.D.)

Staff-Years: 2

Sample Type: Human subjects

Summary of Work: The aim of this project is the development of statistical methods that either take into account interpixel correlation or apply global image transform methods that permit an analysis of uncorrelated image components. Of typical interest is the investigation of differences between either images from individual subjects acquired under different experimental conditions or between average images of subjects from different diagnostic groups. Three different statistical methods have been developed, based on the Fourier transform, the wavelet transform and the theory of Gaussian random fields. In the Fourier domain, the statistics at different wave numbers are uncorrelated and inference tests can be performed unencumbered by spatial correlations. This method provides for rigorous statistical tests with well-known properties and interpretations, but result in spatially uniform image blurring and may yield relatively poor spatial localization. For the wavelet-transform based analysis, a mathematically rigorous theory has been established that applies parametric statistical tests on wavelet coefficients and result in estimates of local image differences by inverse wavelet transform of only significant coefficients. The method provides for good spatial localization and the implementation of locally adaptive image smoothing, but there has not been much experience accumulated for the interpretation of test outcomes and estimates of image differences. Gaussian random field analysis has good spatial localization properties and permits the investigation of correlations with external variables (e.g., age), but it results in spatially uniform image blurring and does not provide for estimates of image differences.

All three methods have been applied to the analysis of PET images from normal and alcoholic subjects and have identified significant differences in generally the same brain regions. Gaussian random field analysis was able to demonstrate, in PET images from alcoholics, a significant negative correlation of glucose utilization in the prefrontal cortex with age. Wavelet-based analysis detected, in fMRI, bilateral blood flow increases in the amygdala due to olfactory stimuli and blood flow increases in the contralateral primary sensorimotor cortex and in focal regions in the ipsilateral cerebellum as a response to a unilateral finger tapping task.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00066-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Psychological and Biological Study of People Who Exhibit Abusive Behavior Patterns

Principal Investigator: D.T. George, M.D. (Staff Clinician)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: M. Linnoila, M.D., Ph.D., LCS
P. Ragan, M.D., LCS
J. Umhau, M.D., LCS
R Rawlings, LCS
M Phillips, LCS
S Graham, LCS
A Cutts, LCS

Collaborating Units: None

Staff-Years: 3

Sample Type: Human subjects (Interviews)

Summary of Work: Domestic violence, involving both children and adults, is a problem of growing national concern. Both our clinical experience and a published report indicate that a significant number of subjects who perpetrate these abusive acts may have an underlying diagnosis of panic disorder. We postulate that the mental state of "being out of control," frequently described by these individuals during the abusive act, is linked to the pathophysiology of panic disorder. To test this hypothesis and to elucidate unique psychological and biological characteristics of patients who perpetrate abusive acts, we will compare subjects who have panic symptoms (e.g., "loss of control") and become aggressive with subjects who are aggressive but do not have panic symptoms and normal controls. These comparisons will consist of psychosocial and family histories, pharmacological challenge studies and a determination of cerebrospinal fluid (CSF) metabolites, as well as a careful analysis of the precipitating events associated with the violence.

Preliminary results show that individuals who initiate acts of domestic violence have a higher than expected prevalence of alcoholism/abuse and panic, borderline personality and obsessive compulsive personality disorders. Results from the lactate challenge paradigm, lying/standing norepinephrine orthostatic challenge, CSF 5-HIAA concentration determination and PET scans all suggest that perpetrators of domestic violence are different from nonviolent controls.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00067-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Psychological and Biological Characterization of Smoking Withdrawal in Alcoholics

Principal Investigator: D.T. George, M.D. (Staff Clinician)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: P. Ragan, M.D., LCS
R. Rawlings, LCS

Collaborating Units: Medical Psychology, Uniformed Services University of the Health Sciences (N. Grundberg, Ph.D.)

Staff-Years: 0.35

Sample Type: Human subjects

Summary of Work: Alcoholics are more likely to smoke cigarettes than individuals without a drinking problem. In this protocol, we are studying neurotransmitter metabolite changes in recently abstinent alcoholics, undergoing nicotine withdrawal, verified by serum nicotine/cotinine concentrations. Alcoholics who continue to smoke or who are nonsmokers, nonalcoholic nonsmokers and smokers make up the comparison groups. The results suggest decreased central noradrenergic turnover during nicotine withdrawal. In addition, there is increased central dopaminergic turnover in nonsmoking alcoholics. Mechanisms underlying these changes and allied changes in other neurotransmitters known to be affected by alcoholism are also being investigated. The results suggest that monoamines may be important in the neurobiological mechanisms of drug addiction.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00092-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Monitoring of Heart Rate Variability During Alcohol Withdrawal Syndrome

Principal Investigator: P. DePetrillo, M.D. (Tenure Track Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.W. Hommer, M.D., LCS

Collaborating Units: None

Staff-Years: 0.6

Sample Type: Human subjects

Summary of Work: The purpose of the project is to examine measures of heart rate variability (HRV), continuously and noninvasively, in male and female alcoholics admitted for drinking cessation. The project serves to test the hypothesis that measures of HRV might serve to distinguish *a priori* sub-groups of patients at risk for more severe alcohol withdrawal in the absence of clinically apparent symptomatology.

The peripheral manifestations of alcohol withdrawal syndrome (AWS) are characterized by rapidly changing autonomic influences. These may vary, minute to minute and hour to hour, depending on the severity and stage of AWS. Consequently, the development of HRV measures, which are relatively insensitive to non-stationarity of signal and could capture relevant data during short periods of measurement, was necessary prior to the protocol implementation.

An algorithm, using phase-space decomposition, was developed that characterizes the type of noise exhibited in time-series measures such as inter-beat interval (IBI). In agreement with other workers using different methodologies, it was found that healthy subjects generate IBI time-series with noise characteristics between noise and noise. The algorithm was found to be sensitive, distinguishing between healthy comparison subjects measured in an eyes-open or eyes-closed paradigm. Relatively short measurement times are required to extract the noise characteristics from the time-series within an acceptable degree of error. Relative insensitivity to non-stationarity of signal was demonstrated. A manuscript outlining this method and its application is in preparation.

The methodology was used to retrospectively analyze the IBI from EKG's obtained from 264 subjects performed under eyes-open and eyes-closed paradigm. Subjects were stratified based on demographic parameters and presence/absence of alcohol dependence. As this data is analyzed, it will provide additional information on the validity and stability of the method. Noise parameters are being compared and contrasted adjusting for age, gender, years of alcohol use and psychopathology. The results of this analysis will provide valuable information regarding population-based variances of the noise parameter.

These results will be applied to the design of the inpatient phase of the study, which will compare and contrast HRV parameters studied in healthy comparison subjects and alcoholics. IBI will be measured in patients for three consecutive 96-hour periods, every 7 days, during the course of treatment. Application of the phase-space algorithm to a heart beat window of 250-350 beats throughout the periods of measurement will allow the analysis of correlations between the noise characteristics and symptoms of AWS. Coarse-graining spectral analysis (CGSA) methodology will also be utilized to simultaneously evaluate parasympathetic and sympathetic nervous system (PNS/SNS) indicators.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00093-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Interaction of Ethyl Alcohol with Cellular Cysteine Proteases

Principal Investigator: P. DePetrillo, M.D. (Tenure Track Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: X. Li, Ph.D., LCS

Collaborating Units: None

Staff-Years: 1.4

Sample Type: Neither human subjects nor tissues

Summary of Work: Certain types of cellular cysteine proteases may play an important role in modulating the activity of G protein-coupled receptors. The activity of this class of enzymes is closely regulated by cytoplasmic and nuclear inhibitors. The interaction of these inhibitors with the target proteases is, in part, mediated by strong hydrophobic interactions. The effects of ethyl alcohol exposure on cysteine protease activity has been studied in cell culture utilizing a PC12 cell line. Activity of these proteases appears to be strongly affected by alcohol exposure.

We have established that exposure of PC12 cells to ethyl alcohol for 96 hours results in a decrease in calcium-stimulated protease activity, which is evident at exposure concentrations of 20 mM; and, a decrease in both isoforms of calpain, which is evident at 40 mM and 80 mM. The results of this work were published (J Neurochem 1997;68:1863-9). An abstract is available at http://www.well.com/user/pdeep/abstracts/pub12ab.html.

We are working towards defining a mechanism for this alcohol-induced inhibition of calcium-activated protease activity. We have generated a polyclonal antibody to Domain IV of calpastatin, a cellular inhibitor of protease activity, and have determined that alcohol exposure increases the levels of calpastatin in PC12 cells. The increased levels of the inhibitor following ethanol exposure may explain the observed decrease in calcium-stimulated calpain activities.

Both calpastatin and the $5HT_3$ receptor are known to be up-regulated by treatment of PC-12 cells with nerve growth factor, suggesting that a common pathway involved in message expression might be operating. Using quantitative RT-PCR, we are determining whether ethanol exposure increases the expression of calpastatin message. We are also examining whether message for the $5HT_3$ receptor, which functions as a calcium-ion gate, is affected by ethanol exposure. A polyclonal antibody for the $5HT_3$ receptor is being raised so that we can determine if protein levels for the receptor are also modulated by ethanol exposure.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00094-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Yohimbine Challenge to Study Noradrenergic Function In Individuals

Principal Investigator: D.T. George, M.D. (Staff Clinician)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: J. Umhau, M.D., LCS
M. Phillips, LCS
S. Graham, LCS

Collaborating Units: None

Staff-Years: 0.9

Human Subjects: Human subjects (Interviews)

Summary of Work: Domestic violence is a major problem affecting society. Results from our research show that individuals who are physically abusive to their significant other frequently experience a hyperaroused state at the time of the violent act which is characterized by increased motor activity, heightened autonomic nervous system activity and accompanied by feelings of "being out of control." This hyperaroused state is similar to that which occurs in post traumatic stress disorder (PTSD). Since PTSD has been associated with changes in noradrenergic function, we postulate that subjects who lose control and are physically violent may also have abnormal noradrenergic function. To test this hypothesis, we have designed a study which compares the behavioral and biochemical effects resulting from the administration of the α2 antagonist, yohimbine, between a group of patients who lose control and are physically violent and comparison subjects.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00225-01 LCS
October 1, 1996 to September 30, 1997

Title of Project: Human Platelet Calcium-Related Signal Transduction Proteins in Alcohol Withdrawal

Principal Investigator: P. DePetrillo, M.D. (Tenure Track Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: A. San Miguel, LCS

Collaborating Units: None

Staff-Years: 1.2

Sample Type: Human subjects and tissues

Summary of Work: Approximately 3% of circulating human platelets, representing the tissues most recently released by megakaryocytes, contain mRNA. Platelets contain mRNA for m-calpain, μ-calpain, calpastatin, and the $5HT_{2a}$ receptor. All of these proteins are involved in a calcium-based signaling pathway.

Having established that certain calcium-related signal transduction proteins appear to be modulated by ethanol exposure in PC12 cells, we are interested in examining whether the level of gene expression in human platelets changes in parallel fashion as that observed in cell culture, during and after exposure to ethyl alcohol.

Our preliminary work was carried out using outdated human platelets. A method was developed, based on RT-PCR, to quantitatively measure the levels of expression for target genes of interest using relatively small quantities of blood (approximately 1 ml). The methodology will be used to probe gene expression in platelets obtained from subjects admitted to our inpatient unit for alcohol detoxification. By sampling the subjects throughout the period of admission, we hope to determine whether significant changes in message occur over time, while relating such changes to relevant demographic parameters, historical data relevant to ethanol consumption and level of alcohol withdrawal symptoms.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00278-08 LCS
October 1, 1996 to September 30, 1997

Title of Project: Behavioral and Physiological Effects of 2-Deoxyglucose Infusions

Principal Investigator: D.T. George, M.D. (Staff Clinician)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: M. Linnoila, M.D., Ph.D. , LCS
J. Umhau, M.D., LCS
M. Phillips, LCS
S. Graham, LCS

Collaborating Units: None

Staff-Years: 0.95

Sample Type: Human subjects

Summary of Work: 2-deoxyglucose (2-DG) is a glucose analog which competitively inhibits glucose-6-phosphate dehydrogenase and leads to intracellular glucoprivation. In previous studies, 2-DG has been used as a stressor to activate the hypothalamic-pituitary-adrenal (HPA) axis, the sympathetic adrenal axis and the appetitive centers of the hypothalamus. Our interest in this paradigm was generated by the clinical observation that alcoholics frequently consume increased amounts of carbohydrates following cessation of drinking. In order to explore possible hypothalamic abnormalities in patients with alcoholism, we administered 2-DG to abstinent alcoholics and measured the resulting behavioral and physiological changes arising from the 2-DG challenge. We postulated that a 2-DG-induced glucoprivic response would give rise to both neuroendocrine and behavioral changes that might elucidate mechanisms of alcohol's action in the hypothalamus. Analysis of the results show alcoholics consume less calories than controls following both 2-DG and placebo administration. Conversely, alcoholics show an exaggerated hypothalamic ACTH response to glucoprivation.

To address the issue of whether this increased response is an acute effect of alcohol withdrawal, the study is now being expanded to include alcoholics who have been alcohol-free for at least six months.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00286-08 LCS
October 1, 1996 to September 30, 1997

Title of Project: Psychobiology of Alcoholism in Women

Principal Investigator: D.T. George, M.D. (Staff Clinician)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: M. Linnoila, M.D., Ph.D., LCS
P. Ragan, M.D., LCS
M. Phillips, LCS
S. Graham, LCS

Collaborating Units: None

Staff-Years: 1.25

Sample Type: Human subjects (Interviews)

Summary of Work: Studies suggest that the pathophysiology of alcoholism may differ significantly between men and women. To explore this possibility, we have employed several pharmacological challenge paradigms to compare the behavioral and endocrine responses between male and female alcoholics. Preliminary analysis of our results show that m-chlorophenylpiperazine (m-CPP) gives rise to a variety of behavioral responses in women alcoholics, ranging from severe anxiety to euphoria. This corresponds to the responses obtained from male alcoholics demonstrated in a previous study. In contrast, very few of the women experienced an m-CPP-induced "desire to drink" response which was found to occur in approximately 40% of the type II (early onset) male alcoholics.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00059-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Determinants of Cognitive Dysfunctions in Neuropsychiatric Disorders

Principal Investigator: H. Weingartner, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.T. George, M.D., LCS
T. Ghiradelli, Ph.D., LCS

Collaborating Units: Univ Strausbourg, France (J. Danion, Ph.D.); Univ Haifa, Israel (S. Breznitz, Ph.D.); Univ College, London (V. Curran, Ph.D.); UCSF (O.Wolkowitz, M.D); UC, Irvine (A.K. Romney, Ph.D.); Colgate Univ (D. Johnson, Ph.D.); Bureau of Labor Statistics (J. Bosley, Ph.D.); BPB, NIMH (R. Post, M.D.); GPB, NIMH (T. Sunderland, M.D.); Lab Personality & Cognition, NIA (P. Costa, Ph.D.); Cognitive Neuroscience, NINDS (J. Grafman, Ph.D.)

Staff-Years: 2

Sample Type: Human subjects

Summary of Work: The aim of this project is to uncover, assess and contrast mechanisms that are responsible for impairments in cognitive functioning in different forms of neuropsychiatric disorders, emphasizing syndromes associated with alcohol abuse. A cognitive neuroscience prospective, along with brain imaging and neuropharmacological methods, are used to consider how disordered cognitive functioning in alcoholics is involved in the development and maintenance of alcohol addiction and abuse. Research studies have been designed to test a set of hypotheses in detoxified alcoholics, which suggest that there are selective impairments in reflective cognitive operations in contrast to data or stimulus-driven cognitive operations, particularly as expressed in problems in inhibition in memory, learning, planning (as in decision processes) and attention (using a new method for exploring different forms of inhibition in attention, i.e., the "attentional blink"). The goals of these studies are to assess and evaluate: a) whether these deficit are independent of other aspects of impaired cognitive functioning in these subjects; b) the neurobiological and behavioral mechanisms of impairments in reflective cognitive operations; c) whether these deficit are relatively unique to alcoholics (i.e., are not observed in patients with other forms of neuropsychiatric disorders); d) conditions that potentiate and attenuate underlying impairments in reflective-inhibitory cognitive functions; e) clinical and therapeutic implications of this type of cognitive impairment; and, f) impairments in cognition that may be antecedents to the development of alcoholism.

We have found that alcoholics demonstrate highly selective impairments in their ability to plan strategically and in learning and remembering and that these subjects show impairments in inhibition such as: errors in remembering; inability to disengage from irrelevant information in attention; inability to track the source of what is remembered; and, tend to be stimulus-driven rather than conceptually-driven in many perception, learning and remembering functions. All of these deficits appear in patients who are unimpaired in most other facets of cognitive functioning. The specific deficits in cognitive functioning apparent in detoxified alcoholics are apparent in both type I and type II alcoholics and are independent of total lifetime consumption of alcohol. The specific deficits in cognitive functioning stand in sharp contrast to the impairments noted in subjects with other forms of neuropsychiatric disorder (such as unmedicated depressed patients) and in normal aging. For example, the impairments in normal aging are generalized across virtually all cognitive domains and, in depressed subjects, there is no evidence of impairment in reflective functions or in inhibition, but an expression of selective deficit in implicit learning and memory, while explicit memory, perception, access to semantic and other forms of knowledge and other cognitive functions are spared.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00060-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Drug Effects on Memory and Related Cognitive Functions

Principal Investigator: H. Weingartner, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.T. George, M.D., LCS
T. Ghirardelli, Ph.D., LCS
D.W. Hommer, M.D., LCS

Collaborating Units: University College, London (V. Curran, Ph.D.); University of Strausbourg (J. Danion, Ph.D.); University of Sussex (T. Duka, Ph.D.); University of British Columbia (E. Eich, Ph.D.); University of California, Irvine (A.K. Romney, Ph.D.); University of California, San Francisco (O.Wolkowitz, M.D.); Colgate University (D. Johnson, Ph.D.); Eli Lilly (S.M. Paul, M.D.); Upjohn (J. Fleishaker, Ph.D.); Geriatric Psychiatry Branch, NIMH (T. Sunderland, M.D.); National Institute on Drug Abuse (S. Heishman, Ph.D.)

Staff-Years: 2

Sample Type: Human subjects

Summary of Work: The studies that make up this project have several related objectives. These include: 1) an understanding of the specific acute and chronic cognitive effects of alcohol; 2) the use of drug probes and models to provide a complementary view of the determinants of learning and remembering from that available utilizing brain imaging techniques; 3) the development of pharmacological models of impaired cognition as expressed in different neuropsychiatric disorders; 4) uncovering cognitive deficits in alcoholics that might ordinarily not be readily apparent but would be expressed under drug challenge conditions. To meet these objectives, normal volunteers, alcoholics and other neuropsychiatric disorder patients are studied in repeat-measure, crossover designs using several contrasting classes of compounds including benzodiazepines, serotonergic drugs, drugs that affect the cholinergic nervous system and those that interact with the N-methyl-D-aspartate (NMDA) receptor. Previously validated cognitive neuroscience methods are used to assess and contrast the effects of these agents in different study populations.

Detoxified alcoholics, treated with benzodiazepines (which mimics many of the effects of alcohol), demonstrate a dramatic impairment in reflective cognitive functioning and inhibitory functions. This form of impairment is not nearly as apparent under placebo test conditions and, furthermore, this deficit is not secondary to changes in many other cognitive domains such as those involved in learning and remembering. In addition, access to what is in knowledge memory is qualitatively different for detoxified alcoholic subjects under benzodiazepine test conditions (compared to placebo), which is not the case in normal controls. These findings provide some new perspective, in cognitive terms, of the ways that alcohol alters mental functions in alcoholics that is distinguishable from the effects of alcohol in normals; as such, it has important clinical implications.

Parallel studies have explored the role of awareness in memory using benzodiazepines as a tool for altering cognitive functioning. Other studies have been designed to examine whether only some aspects of attentional functioning are impaired following alcohol administration in normals while other facets of attention are spared. This research has the dual goal of utilizing drug probes for exploring the differentiated nature of attention (as well as learning and memory), while at the same time providing new and clinically relevant information about the cognitive effects of alcohol. These attentional-cognitive studies are designed to contrast the effects of alcohol on goal-directed (or top-down) control processes with stimulus-driven (or bottom-up) control functions. Top-down cognitive functions are the types of operations that are impaired in alcoholics.

We have also demonstrated that the effects of other psychoactive agents, such as drugs that affect the NMDA receptor, mimic the cognitive changes expressed in normal aging, but not that of amnesia or dementia, in both young and elderly normal controls. Ketamine also potentiates the cognitive response to both benzodiazepines and cholinergic antagonists, such as scopolamine. In general, the cognitive effects of each of these classes of drugs are largely independent of their sedative effects. In a study of early- and middle-stage Alzheimer disease patients, we were unable to demonstrate positive cognitive effects of stimulant treatments despite successful behavioral activation. This confirms our previous findings which demonstrated a dissociation between mood and stimulant effects of several drugs and their cognitive effects, including effects on learning, memory, attention and perception.

Our studies have provided a clearer picture of the specific cognitive deficits that are apparent in alcoholics and the impact of drugs, similar to alcohol, on the cognitive functioning of the alcoholic. These findings are important in understanding the mechanisms that account for normal memory as well as forms of impaired memory functions. This research also serves as a basis for the development of drug and behavioral treatments of cognitively impaired patients, particularly those suffering from addictions to alcohol and other psychoactive drugs.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00068-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: CNS Serotonin and the Regulation of Peripheral Glucose Metabolism

Principal Investigator: R.L. Eskay, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda, MD 20892-1256

Other Personnel: M. Linnoila, M.D., Ph.D., LCS
M. Torda, Ph.D., LCS

Collaborating Units: None

Staff-Years: 1.5

Sample Type: Neither human subjects nor tissues

Summary of Work: Increased alcohol preference and consumption, depressed mood, and impulsive aggression are thought to be linked, in part, through decreased central serotoninergic (5HT) activity. In agreement with this postulate, certain agents which increase central serotonergic neurotransmission (5HT precursors, 5HT uptake inhibitors, 5HT receptor antagonists) attenuate ethanol intake, improve memory function in intoxicated patients and may improve memory functions in patients with Korsakoff's psychosis. Recently, a possible pattern of atypical glucose metabolism has emerged in alcohol abusing, impulsive, violent offenders with apparent central serotonergic dysfunction. In a group of impulsive offenders, hypoglycemia was possibly due to increased insulin secretion. It is possible that a relative hypoglycemic state, or abnormal insulin levels, may contribute to violent, aggressive behavior in violent offenders with apparently reduced 5HT activity; however, this hypothesis awaits substantially more scientific verification. Although appropriate animal studies have not been performed which demonstrate a cause and effect relationship between altered 5HT activity and abnormal glucose metabolism, there is overwhelming evidence that appropriate glucose levels are maintained through a complex feedback system which involves the sympatho-adrenalmedullary system through the glucose mobilizing hormone, epinephrine, and the endocrine pancreas via insulin and glucagon secretion. Finally, we have established that serotonergic neurotransmission, particularly events which are mediated via the central $5HT_{1a}$ receptor subtype, is a significant mediator of normal glucose homeostasis.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00069-06 LCS
October 1, 1996 to September 30, 1997

Title of Project: Na+, K+-ATPase:Function and Regulation, Alcoholism, Neuroscience

Principal Investigator: M. Linnoila, M.D., Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: T. Foley, Ph.D., LCS

Collaborating Units: None

Staff-Years: 1.5

Sample Type: Neither human subjects nor tissues

Summary of Work: We have been investigating the regulation of Na+, K+-ATPase and associated Na+/K+-pump activities in synaptosomal preparations from rat brain. We have identified a novel substoichiometric mode of synaptosomal Na+, K+-ATPase (and associated Na+/K+-pump) inhibition by very low concentrations (IC_{50}=10-15 M) of the specific steroid ligand, ouabain. Substoichiometric inhibition by ouabain is transient and is observed under conditions that favor the Na+ and ATP-binding conformer (E1) of the enzyme. In addition, substoichiometric inhibition apparently involves a protein kinase A (PKA) and cyclo-oxygenase-dependent production on a secondary Na+, K+-ATPase inhibitor that may be a cyclooxygenase-derived oxidant. Furthermore, ouabain structure-activities have led to the identification of the reduced lactone derivative of ouabain (dihydroouabain) as an antagonist of the substiochiometeric inhibition by ouabain. We have found that the low concentrations (IC_{20}= 5-10 mM) of ethanol also inhibit synaptosomal Na+, K+ ATPase and Na+/K+-pump activity via the PKA and cyclooxygenase-dependent mechanism. This novel mode of Na+, K+-ATPase inhibition by ethanol is evident under depolarizing conditions (i.e., veratridine) such that the depolarization-dependent activation of the synaptosomal Na+/K+-pump is inhibited by 10-25 mM ethanol. Importantly, the inhibition by ethanol was also found to be antagonized by dihydroouabain. We are currently modifying the structure of dihydroouabain to promote its brain uptake so that the importance of Na+, K+-ATPase inhibition, as a possible mediator of the CNS actions of ethanol, can be delineated.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00077-04 LCS
October 1, 1996 to September 30, 1997

Title of Project: CNS Serotonin Activity, Tolerance, Anesthesia, and PET Scans in Rhesus Macaques

Principal Investigator: J.D. Higley, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Poolesville MD 20837

Other Personnel: D.W. Hommer, M.D., LCS
M. Linnoila, M.D., Ph.D., LCS
B. Roberts, Ph.D., LCS
S.E. Shoaf, Ph.D., LCS
K. Weld, M.S., LCS
G. Flory, LCS
A. Hurley, LCS
G. Pushkas, LCS

Collaborating Units: Laboratory of Comparative Ethology, NICHD, NIH (S. Suomi, Ph.D.; S. Lindell; C. Shannon; T. Tsai; K. Zajicek)

Staff-Years: 2

Sample Type: Neither human subjects nor tissues

Summary of Work: During the past year, our research has continued investigation of central nervous system (CNS) serotonin correlates of low CSF 5-HIAA and tolerance to the intoxicating effects of alcohol. To assess the role of impaired serotonin functioning and intrinsic tolerance on excessive alcohol consumption and the related high levels of aggression, CSF was obtained from alcohol naive rhesus macaque subjects and assayed for CSF 5-HIAA concentrations. Subjects were then dispensed identical doses of IV alcohol and rated for levels of intoxication. Following the ratings for intoxication, subjects were allowed to consume an alcohol solution. (1) Subjects with lower ratings for intoxication and low CSF 5-HIAA were more likely to consume alcohol to excess. (2) Peer-reared monkeys (monkeys reared without adults, with only age-mates present) were also more likely to consume alcohol to excess. (3) Rates of aggression, measured while the subjects were intoxicated, were correlated with lifetime rates of severe aggression which suggests that high rates of aggression during intoxication are an extension of a lifelong pattern of severe aggression rather than a special form of aggression. (4) As an extension of these findings, interindividual differences in CSF 5-HIAA concentrations were also shown to correlate with time to recover from ketamine anesthesia. (5) Subjects with low CSF 5-HIAA concentrations also showed high serotonin transporter binding, as measured by β-CIT uptake in SPECT imaging. High β-CIT uptake was correlated with high rates of aggression and minimal intoxication following a modest dose of alcohol. (6) Studies were initiated that investigated neuroanatomical differences in frontal serotonin functioning in monkeys with low or high CSF 5-HIAA concentrations. (7) PET studies continued to investigate the underlying serotonin synthesis rates in subjects with low and high CSF 5-HIAA concentrations.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00078-01 LCS
October 1, 1996 to September 30, 1997

Title of Project: The Behavioral and Neurochemical Basis of Animal Models of Alcoholism

Principal Investigator: M. Linnoila, M.D., Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: F.S. Hall, Ph.D., LCS
G. Fong, LCS

Collaborating Units: National Institute of Mental Health (A. Pert, Ph.D.)

Staff-Years: 0.75

Sample Type: Neither human subjects nor tissues

Summary of Work: This protocol will examine several animal models of alcoholism in rats: isolation-rearing, the Fawn hooded strain and the effects of 5,7-DHT lesions of the serotonin systems. This project continues previous work which was supported by a National Research Council Research Associate (FSH). In these models, behavioral and neurochemical examinations of subjects will be made as well as examination of responses to ethanol. In particular, differences in the effects of serotonin and dopamine responses to ethanol will be examined using *in vivo* microdialysis to measure the brain levels of these neurotransmitters. In addition, the α-methyltryptophan method will be evaluated as a technique for examining central serotonin function.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00079-04 LCS
October 1, 1996 to September 30, 1997

Title of Project: Psychobiology of Antisocial Behavior and Health

Principal Investigator: J.D. Higley, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Poolesville, MD 20837

Other Personnel: L. Akhtar, LNG
D. Goldman, M.D., LNG
M. Linnoila, M.D., Ph.D., LCS
D.A. Nielsen, Ph.D., LNG
S.E. Shoaf, Ph.D., LCS
K. Weld, M.S., LCS
G. Flory, LCS
A. Hurley, LCS
G. Pushkas, LCS
S. Graham, LCS

Collaborating Units: Laboratory of Comparative Ethology, NICHD, NIH (S. Lindell; C. Shannon; S. Suomi, Ph.D.; T. Tsai; K. Zajicek); Laboratory Animal Breeders Services, Yemassee, SC (P. Mehlman, Ph.D.; D. Taub, Ph.D.; B. Fernard)

Staff-Years: 5.0

Sample Type: Neither human subjects nor tissues

Summary of Work: During the past year, our research included studies of: (1) neurobiology and behaviors that laboratory work has shown are correlated with excessive alcohol consumption, including aggression, impulsivity, sleep deficits and reduced affiliative social behavior; (2) genetic influences on low cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) concentrations; (3) traits maintaining the low CSF 5-HIAA genotype in the gene pool; (4) two closely related macaques with known differences in aggressiveness and sociality. Comparisons were made between and within species for differences in CSF 5-HIAA concentrations and corresponding behavior. Data showed that species with lower CSF 5-HIAA concentrations were more aggressive and less prosocial. Within-species differences also showed a negative correlation between CSF 5-HIAA concentrations, aggression and social isolation; (5) assessment of naturally occurring male social dominance and its relationship to CSF 5-HIAA concentrations; (6) new technologies were used, including telemetry for assessment of diurnal activity, and new traps for capture of subjects.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00095-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Isolation and Characterization of Sulfonylurea-Like Compounds and Insulin Release

Principal Investigator: M. Torda, Ph.D. (Adjunct Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: R.L. Eskay, Ph.D., LCS
M. Linnoila, M.D., Ph.D., LCS

Collaborating Units: None

Staff-Years: 1.5

Sample Type: Neither human subjects nor tissues

Summary of Work: Selected serotonergic compounds, through a CNS site of action, appear to be able to release sulfonylurea-like peptides from the pituitary gland. The therapeutic significance of the sulfonylurea compounds in the treatment of type II diabetes for regulating plasma glucose levels has been recognized for decades without a full appreciation of the mechanism of action of these compounds, nature of the receptor or the demonstration of the existence of endogenous ligands. After extensive isolation and characterization of sulfonylurea-like activity in the CNS and pituitary gland, several peptide fractions have emerged which: (1) enhance insulin secretion, (2) inhibit sulfonylurea drug binding to sulfonylurea receptors, and (3) appear to mimic the electrophysiological membrane changes observed with sulfonylurea drugs. A novel peptide and a known peptide, with a novel function, have emerged from these studies. It is envisioned that there exists a family of novel endogenous peptides which modulate sulfonylurea receptors. Studies designed to elucidate these peptides and their physiological role in glucose homeostasis are ongoing.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00258-13 LCS
October 1, 1996 to September 30, 1997

Title of Project: Violent Behavior, Neurotransmitters, Glucose Metabolism and Alcohol Abuse

Principal Investigator: M. Linnoila, M.D., Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG

Collaborating Units: Department of Psychiatry, University of Helsinki (M. Virkkunen, M.D.)

Staff-Years: 0.4

Sample Type: Human subjects

Summary of Work: We have investigated neurotransmitter metabolites and glucose metabolism in incarcerated violent offenders, arsonists and healthy volunteers. We have found that low cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) concentrations and hypoglycemia, during oral glucose tolerance tests, are associated with each other and impulsive violent acts and fire setting. To elucidate the pathophysiology of the reactive hypoglycemia, englycemic insulin clamp studies with indirect calorimetry have been performed on more than 70 offenders and healthy volunteers. The data have not been analyzed, because initial power calculations indicated that a larger sample size is necessary. Sample collection for the molecular genetic family study on alcoholic, violent offenders has been completed. The clinical data have been blind rated for psychiatric diagnoses and all biochemical and physiological data have been entered in the computers and double checked for errors. Molecular genetic analyses are underway. Thirteen hundred DNA samples, which represent a random sample of Finns and are without identifiers, were received from Dr. Leena Peltoven in the National Public Health Institute, Helsinki. Together with our own control sample, which consists of 300 volunteers, these samples will be used to establish the true allelic frequencies of rare polymorphisms among the Finnish population.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00277-09 LCS
October 1, 1996 to September 30, 1997

Title of Project: Nonhuman Primate Models of Alcohol Consumption and Excessive Aggression

Principal Investigator: J.D. Higley, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Poolesville MD 20837

Other Personnel: L. Akhtar, LNG
D. Goldman, M.D., LNG
M. Linnoila, M.D., Ph.D., LCS
D.A. Nielsen, Ph.D., LNG
S.E. Shoaf, Ph.D., LCS
K. Weld, M.S., LCS
G. Flory, LCS
A. Hurley, LCS
G. Pushkas, LCS
S. Graham, LCS
N. Salem, Ph.D., LMBB
J. Hibbeln, M.D., LMBB

Collaborating Units: Lab Comparative Ethology, NICHD, NIH (S. Lindell; C. Shannon; S. Suomi, Ph.D.; T. Tsai; K. Zajicek); University of California, Dept Psychiatry (R. Poland, Ph.D.); University of Michigan (J. Woods, Ph.D.)

Staff-Years: 7.5

Sample Type: Neither human subjects nor tissues

Summary of Work: During the past year, our research included: studies of etiological mechanisms (i.e., genetic and environmental influences) on low CSF 5-HIAA concentrations and alcohol consumption; studies of behaviors associated with individual differences in alcohol consumption in rhesus monkeys selectively bred for target CSF 5-HIAA concentrations; investigations of variables that affect alcohol consumption (e.g., whether the capacity to binge or sip an alcohol solution affects rates of consumption in impulsive monkeys); pharmacological treatment of aggression and alcohol consumption using tryptophan and serotonin reuptake inhibitors; tryptophan treatment of self-injurious behavior; investigations of the role taste plays in excessive consumption and whether an affinity for reinforcing agents such as sugar solutions are positively correlated with high alcohol consumption; studies of sleep and activity in subjects with low CSF 5-HIAA; studies of effect of rearing on fear and anxiety, the acquisition of social dominance and subsequent adult maternal behavior; development of new technologies to study chronic alcohol consumption. In collaboration with Dr. Woods, studies were initiated that investigate the role that gustatory factors play in alcohol consumption and he began investigating whether high alcohol consumption is correlated with excessive intake of opiates. A collaboration was initiated with LMBB to investigate the role of EFA in CNS serotonin functioning. As a corollary of this investigation, a laboratory-wide assessment of cholesterol and EFA were made to correlate with CSF 5-HIAA concentrations.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00287-07 LCS
October 1, 1996 to September 30, 1997

Title of Project: Stress Axis, Activation, Site-Specific CNS Neurodegeneration and Ethanol

Principal Investigator: R.L. Eskay, Ph.D. (Senior Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: M. Torda, Ph.D., LCS
C. Hamelink, LCS

Collaborating Units: None

Staff-Years: 1.5

Sample Type: Neither human subjects nor tissues

Summary of Work: Consumption of ethanol (Et) alters certain regulatory aspects of the hypothalamic-pituitary-adrenal (HPA) axis. Because the integrity of this system depends on the coordinated synthesis and secretion of specific regulatory substances at the hypothalamic (e.g., corticotropin-releasing hormone (CRH); vasopressin (AVP); biogenic amines), pituitary gland (e.g., β–endorphin; ACTH) and adrenal gland (e.g., catecholamines; glucocorticoids) level, we have been evaluating the impact of Et at each level of the HPA axis. Activation of the HPA axis, or hypercortisolism, accompanies both short- and long-term consumption of Et and the Et withdrawal syndrome. Alcoholics often present with a pseudo-Cushing's syndrome in which 17-40% do not respond to the dexamethasone suppression test during the first week of abstinence. Since a relative state of elevated glucocorticoids (chronic continuous or chronic intermittent) can lead to neural changes and even cell death, particularly in the hippocampus, the progressive loss of cognitive capacity in many alcoholics may indeed be due, in part, to hypercortisolemia and subsequent irreversible neural damage in the hippocampus and other areas of the CNS.

Using an intragastric cannulated rodent model and short-term (4 days) intermittent alcohol administration, we have demonstrated site-specific CNS neurodegeneration in the dentate gyrus of the hippocampus, the entorherial cortex and the piriform cortex. Assessment of the role of increased glutamatergic neurotransmission, Ca++ uptake and levels of glucocorticoids in the observed neurodegeneration continues with hormonal and pharmacological replacement therapy.

This project was formerly titled "Stress Axis, Immune System-Derived Cytokines and Ethanol."

INTRAMURAL RESEARCH PROJECT ZO1 AA 00096-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Protein Turnover in the Endoplasmic Reticulum

Principal Investigator: S.E. Shoaf, Ph.D. (Tenure Track Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: B. Roberts, Ph.D., LCS

Collaborating Units: None

Staff-Years: 0.5

Sample Type: Neither human subjects nor tissues

Summary of Work: We have been investigating the mechanisms by which membrane proteins are turned over in the endoplasmic reticulum. Attention has been focused on CYP2E1, a P-450 enzyme that is induced by alcohol and is thought to enhance lipid peroxidation and thereby liver damage during alcohol consumption. Antigen processing and degradation of this protein and other related forms are mediated by a common mechanism. We sought to determine whether the balance between the production of antigenic peptides and proteolytic processing in the endoplasmic reticulum were mutually dependent events. Our findings have been presented in two articles (J Biol Chem 1997;272:9771-8 and Roberts et al: J Biol Chem, submitted).

Project is now terminated.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00097-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Comparative Ethanol Metabolism in Normal Volunteers

Principal Investigator: S.E. Shoaf, Ph.D. (Tenure Track Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.T. George, M.D., LCS
D. Goldman, M.D., LNG
E. Singley, LCS
J. Wan, Ph.D., LCS

Collaborating Units: Clinical Center, NIH (R. White, R.N., M.S.)

Staff-Years: 2

Sample Type: Human subjects

Summary of Work: The incidence of alcohol-related disease is different in different populations. The allelic frequency of the enzymes thought to be primarily responsible for alcohol metabolism is also different in these populations. Alcohol distributes in total body water, therefore, doses of alcohol should be made on a g/l of total body water basis instead of a g/kg basis. Previous attempts to compare ethanol metabolism in different ethnic groups did not compare individuals of similar genotype or adjust the dose to account for possible differences in total body water.

The protocol has been approved, assays developed and patient accrual has begun.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00098-02 LCS
October 1, 1996 to September 30, 1997

Title of Project: Distribution of Serotonergic Neurons Using ^{11}C-Compounds and PET Imaging

Principal Investigator: S.E. Shoaf, Ph.D. (Tenure Track Investigator)
LCS, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D.W. Hommer, M.D., LCS
W. Williams, M.D., LCS

Collaborating Units: NIH, PET (R. Carson, Ph.D.; P. Baldwin; P. Herscovitch, M.D.; B. Schmall, Ph.D.); National Center for Research Resources, NIH (M.Thomas, D.V.M.)

Staff-Years: 1

Sample Type: Neither human subjects nor tissues

Summary of Work: Twelve monkeys were scanned following administration of methyltryptophan. Six monkeys were scanned twice due to low specific activity administered with the first dose. A paper has been written and submitted.

In two monkeys, dosimetry studies for [^{11}C] MDL-100907 have been done and are being evaluated. Binding studies are being performed on several fluoro-derivatives of WAY-100635 and MDL-100907.our understanding of the teratogenic actions of ethanol and the chances for effective treatment.

FY 1997 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1996 - 30 SEPTEMBER 1997)

LABORATORY OF MEMBRANE BIOCHEMISTRY & BIOPHYSICS

NORMAN SALEM, JR., Ph.D., CHIEF

DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
NATIONAL INSTITUTES OF HEALTH

SYNOPSIS

LABORATORY OF MEMBRANE BIOCHEMISTRY & BIOPHYSICS

INTRODUCTION

This is the first reporting period in which the LMBB has functioned as a physically united laboratory, now that various Section relocations and installation of principal equipment have been completed. Difficulties with the physical plant at the Flow Building continue and the newly renovated animal space continues to be unusable for animal holding. The Laboratory has undergone review by the Board of Scientific Counselors and both the investigators and the research projects received outstanding evaluations. We look forward to increased scientific progress and productivity as the Laboratory begins to function as an integrated unit.

OFFICE OF THE CHIEF

Our laboratory has taken the worldwide lead in investigating *in vivo* essential fatty acid (EFA) metabolism in humans and large animals. A study of normal volunteers, smoking controls and alcoholic smokers has been completed. Of significance are the qualitative findings that humans are capable of the *in vivo* conversion of linoleate (18:2n6) to arachidonate (20:4n6) and of linolenate (18:3n3) to docosahexaenoate (22:6n3). When subjects' diets were changed to increase intake of long chain polyunsaturates, there was a decrease in the accretion of deuterated 20- and 22-carbon polyunsaturates in their plasma, suggesting that there was a negative feedback loop of the polyunsaturates operating on their biosynthesis. It appeared that 20:5n3 was a powerful regulator of 20:4n6 synthesis, as well. It is of great significance that alcoholic EFA metabolism appears to be greater than that of normal volunteers or smokers, particularly with regard to 22:6n3. Absorbtion of the fatty acid labels was equal or greater in alcoholics. The isotopic enrichment of the EFA metabolites, like 22:6n3 from its precursor, 18:3n3, was greater in alcoholics, suggesting that anabolic processes are increased. This is in contrast to the commonly held notion that alcohol inhibits fatty acid metabolism via its effects on desaturases. The concept that has been developed, and gaining support from our studies, is that alcohol increases fatty acid turnover and catabolism leading to increases in anabolic processes. Acute alcohol challenges can lead to stimulated metabolism and higher tissue concentrations of lipids containing 22:6n3. Conversely, chronic alcohol abuse leads to an inability of the stimulated anabolic processes to maintain tissue levels of 20:4n6 and 22:6n3 due to the oxidative insult of frequent, high alcohol blood levels. The loss of liver and brain 22:6n3 is believed to lead to organ damage and failure, when coupled with the direct toxic effects of alcohol. Prevention of the loss of organ 22:6n3, by means of increased dietary intake, is suggested as a therapeutic approach for alcoholics.

These findings, that humans biosynthesize 20:4n6 and 22:6n3, were confirmed in newborn infants in a collaborative study with Ricardo Uauy in Chile. Infants, in the first week of life, who were of a gestational age of 28-40 weeks, were capable of converting 18:2n6 to 20:4n6 and 18:3n3 to 22:6n3 *in vivo*. An estimation of the total amounts of these products accreted during the six-day experiment indicated that the metabolic supply is inadequate for the structural requirements of neuronal formation. The increase in the mass of brain 20:4n6 and 22:6n3 is much greater than the amount of these EFA being formed at this developmental stage. It was observed that the more premature infants appeared to express a higher EFA metabolic capacity towards 22:6n3 than term infants. Also, infants who were small for their gestational age (SGA) possessed a lower EFA metabolic capability.

The development of animal models, in which the alcohol- or dietary-insufficiency induced loss of neural 22:6n3 occurred, underwent rapid progress. Rhesus monkeys, after 4 years of ad lib alcohol drinking in combination with a diet low in EFA, exhibited evidence of liver disease. There was steatosis as well as perivenular and sinusoidal fibrosis in most animals. This model of alcoholic liver disease (ALD) employs a lower amount of alcohol (a mean level of 24%

of calories, range 16-42%) relative to most other studies in which comparable liver pathology was observed. This difference was attributed to the difference in diet, which was designed to contain lower amounts of EFA, particularly of the n3 class, as well as moderate amounts of vitamins C and E. It was demonstrated that there were losses of 20:4n6 and 22:6n3 in plasma, lipoproteins, erythrocytes and liver. Alcohol exposure was not altering EFA absorption or elongation/desaturation as stable isotopically labeled 18:2n6 and 18:3n3 metabolism was not decreased *in vivo* in the animals consuming alcohol. However, products of fatty acid peroxidation, such as hydroxynonenal and isoprostanes, were markedly elevated in the alcohol consuming group, suggesting a general increase in lipid oxidative metabolism. These data are consistent with our concept that a diet low in EFA is a predisposing factor for alcohol liver disease.

Alterations in fatty acid levels in fetal alcohol syndrome (FAS) have been studied in cat models and in human infants, the latter in collaboration with the Fetal Alcohol Research Center, Wayne State University. In cord blood obtained from human infants exposed to alcohol *in utero*, there was a significant increase in the percentage of plasma 22:6n3. In a rat model of FAS, when alcohol exposure was interrupted for one day, there was a loss of brain 22:6n3 but an increase in plasma 22:6n3, as was found in human infants. Overall, when the data are expressed in terms of concentration, there was a decrease in plasma fatty acid content and an increase in the n6 catabolic product, 4-hydroxynonenal. In domestic cats, exposed to alcohol from approximately day 14 of pregnancy until term and in which the mothers were subjected to a low EFA diet (3 wt% corn oil), there were marked effects of alcohol on newborn kitten survival and morphology. In the alcohol-exposed kittens, where the photopic α-wave was measured at 8 weeks of age, the α-wave latency was significantly shorter and the photopic α-wave amplitude higher than those of control animals. The photopic β-wave amplitude was greater in the alcohol-exposed kittens, as well.

It was found that plasma levels of EFA correlate with the levels of neurotransmitter metabolites in cerebrospinal fluid (CSF). The serotonin metabolite, 5-HIAA, correlated with plasma 20:4n6 in healthy volunteers, but with 18:3n3 in late-onset alcoholics and with 22:6n3 (negatively) in early-onset alcoholics. Similar correlations for the dopamine metabolite, homovanillic acid (HVA), were observed in healthy volunteers and early-onset alcoholics. In healthy volunteers, there was a direct correlation between 22:6n3 levels and 5-HIAA, however, in early-onset alcoholics, there was a strong inverse correlation ($r=-0.38$, $p<0.0002$). Since there is a well-known correlation of impulsive violence, suicide and depression associated with low levels of CSF 5-HIAA, plasma levels of EFA may be predictive of these psychiatric variables.

SECTION OF FLUORESCENCE STUDIES

Among the research goals of this Section are the determination of the structure-function relationship associated with the requirement of long chain polyunsaturated acyl chain phospholipids in the nervous system and retina and receptor function. Many neuro-transmitter receptors and all visual pigments are members of the G protein-coupled receptor family and reside in membranes containing very high levels of polyunsaturated acyl chains, particularly those in the n3 series, such as 22:6n3. Deficiencies in n3 acyl chains have been shown to lead to impaired learning and visual responses. These studies employ the rod photoreceptor visual transduction pathway, which utilizes rhodopsin as the receptor, as a model for G protein-coupled receptor pathways. In addition to studies of the role of membrane lipid composition in modulating receptor function, the effect of ethanol on these transduction pathways is also being studied. The extent of formation of metarhodopsin II (MII), the activated form of rhodopsin with respect to G protein activation, is being studied as a functional measure of pathway activity. Parallel studies of acyl chain packing properties of the phospholipid bilayer are being carried out using time-resolved fluorescence spectroscopy and differential scanning calorimetry (DSC). Isolation of purified rhodopsin and reconstitution into bilayers of defined lipid composition has allowed an evaluation of the role of phospholipid acyl chain composition.

These studies have identified acyl chain packing free volume as a compositionally dependent membrane property which modulates rhodopsin activation. Increasing acyl chain

polyunsaturation is seen to both increase free volume and enhance rhodopsin activation. In both mixed chain saturated-unsaturated phospholipids and diunsaturated phospholipids, molecules with 22:6n3 acyl chains are most effective in promoting rhodopsin activation. Cholesterol has been shown to decrease free volume and inhibit rhodopsin activation. Our studies show that acyl chain unsaturation buffers the effect of cholesterol in inhibiting rhodopsin activation and that lipids containing 22:6n3 are the most effective in opposing the action of cholesterol. All the effects we have observed can be explained in terms of a novel domain model of lipid acyl chain packing in bilayers that was developed in this laboratory. This model describes lateral domain heterogeneity in lipid distribution in the plane of the membrane and provides a conceptual framework for explaining the unique structural features of membranes containing 22:6n3 and the dependence of rhodopsin activation on acyl chain unsaturation and bilayer cholesterol content. In studies of the effect of ethanol on rhodopsin activation, we have shown that ethanol increases bilayer free volume and also stimulates rhodopsin activation. The effects of ethanol are more pronounced in polyunsaturated lipid bilayers than in more saturated membranes. These latter studies demonstrate a lipid component to the mechanism of action of ethanol in potentiating the activation of the G protein-coupled receptor, rhodopsin.

SECTION OF MASS SPECTROMETRY

It has been proposed that an important mechanism underlying many of the effects of ethanol is its capacity to alter polyunsaturate metabolism. The principal objective of our research is to elucidate biological and metabolic functions of polyunsaturated fatty acids, docosahexaenoic acid (22:6n3) and arachidonic acid (20:4n6), in the nervous system with particular reference to their modulation by ethanol. During this period, we found that polyunsaturates affect the survival of neuronal cells and the accumulation of phosphatidylserine (PS), which is thought to be involved in growth factor signaling leading to cell survival.

Polyunsaturated fatty acids, 20:4n6 and 22:6n3, prevented the apoptotic cell death of both Neuro 2A and PC-12 cells, assessed by DNA fragmentation and by Hoechst staining. While arachidonic acid (up to 25 μM) protected these cells from apoptosis induced by serum deprivation after short-term exposure, the protection by 22:6n3 against apoptotic neuronal cell death occurred only after the incorporation of 22:6n3 into phospholipids. After a prolonged incubation, this fatty acid was accumulated mainly in aminophospholipids, PE and PS, suggesting that 22:6n3 as a membrane phospholipid constituent, especially as amino-phospholipids, may be important for the protective effect. The analysis of phospholipid molecular species from n3-deficient animal tissues, by electrospray liquid chromatography/ mass spectrometry (LC/MS), revealed that the accumulation of polyunsaturated PS was modulated by the 22:6n3 status and by ethanol. From the serine base exchange reaction, using both n3-deficient animal model and polyunsaturated fatty acid enriched C-6 cell model, we found that 22:6n3 promotes PS biosynthesis while ethanol exerts the opposite effect. Other polyunsaturated fatty acids, such as 22:5n6 or 20:4n6, could not fully support the biosynthesis of polyunsaturated PS. Taken together, the biological significance of 22:6n3 may reside with membrane phospholipids, at least partly, through the modulation of poly-unsaturated PS biosynthesis and accumulation. The loss of polyunsaturated PS, caused either by depleted 22:6n3 supply or by ethanol abuse, may have significant implications for neuronal dysfunction.

We also found that basal melatonin synthesis was significantly increased with n3-deficiency with concomitant decrease in free fatty acids and 12-lipoxygenase products. These data suggest that polyunsaturated fatty acids affected the pineal function which may in turn influence the synchronization of biological rhythms. In a continuing effort to develop a sensitive technique to analyzed trace neurosteroids from biological fluids, a GC/MS/NCI technique, which can detect 1 pg levels of several neurosteroids, was developed using derivatives of electron capturing moieties. Characterization of the level of neuroactive steroids in rat brain and plasma as well as in monkey CSF is now in progress using this method.

2H NMR on deuterated lipids yields an order parameter profile of lipid hydrocarbon chains which has been shown to be very sensitive to changes in membrane thickness and area. The measurement of deuterium order parameters has an intrinsic time scale of 10-5 s. Over this period of time, all lipid molecules sample every chain conformational state available in a typical bilayer, resulting in well resolved NMR signals. Improvements in NMR spectrometer performance and data analysis, using software written within the Section, have enhanced precision and reproducibility of order parameter determination. We are able to measure chain order parameters with a resolution of DS = ±0.001, which corresponds to changes in bilayer hydrophobic thickness as small as ±0.1 Å and changes in lipid area per molecule as small as ±0.2 Å2. The method is well-suited to detect small changes in membrane organization which are relevant for protein function. The elastic area compressibility modulus, Ka, of lamellar liquid crystalline bilayers was determined by this new experimental approach using 2H NMR order parameters of lipid hydrocarbon chains together with lamellar repeat spacings measured by x-ray diffraction. The combination of NMR and x-ray techniques yields accurate determination of lateral area per lipid molecule. Samples of saturated, monounsaturated and polyunsaturated phospholipids were equilibrated with polyethylene glycol (PEG) 20,000 solutions in water at concentrations from 0 to 55 wt% PEG at 30°C. This procedure is equivalent to applying 0 to 8 dyn/cm lateral pressure to the bilayers. The resulting reductions in area per lipid were measured with a resolution of ±0.2 Å2 and the fractional area decrease was proportional to applied lateral pressure. For di-14:0 PC, 18:0d35-18:1n9 PC and 18:0d35-22:6n3 PC cross-sectional areas per molecule in excess water of 59.5, 61.4 and 69.2 Å2, and bilayer elastic area compressibility moduli of 141, 221 and 121 dyn/cm were determined, respectively. Combining NMR and x-ray results enables the determination of compressibility differences between saturated and unsaturated hydrocarbon chains. In mixed-chain 18:0d35-18:1n9 PC, both chains have similar compressibility moduli, however, in mixed-chain polyunsaturated 18:0d35-22:6n3 PC, the saturated stearic acid chain appears to be far less compressible than the polyunsaturated docosahexaenoic acid chain.

The NMR section of LMBB is developing magic angle spinning (MAS) techniques for the study of models and biomembranes. With special inserts for 4 mm MAS rotors, developed in collaboration with the mechanical shop at NIH, we are able to spin membrane samples at speeds up to 15 kHz and routinely achieve a resolution for lipid proton NMR signals of 20 Hz or better. MAS NMR allows investigation of lipids in model as well as biological membranes containing proteins. It does not require membrane perturbing preparation procedures like sonication and has better sensitivity than high-resolution NMR experiments on sonicated lipid dispersions. The location of an ethanol molecule within a membrane, an issue of considerable controversy, was investigated directly by NMR with two-dimensional NOESY. Lipid and ethanol 1H NMR resonances of multilamellar liposomes were resolved by MAS. We observed strong proton lipid-ethanol crosspeaks in dispersion of saturated di-14:0 PC and monounsaturated 18:0d35-18:1n9 PC and in polyunsaturated 18:0d35-22:6n3 PC. Crosspeak intensity has been interpreted in terms of an ethanol distribution function over the lipid bilayers. Ethanol resides with the highest probability at the lipid water interface near the lipid glycerol backbone and upper methylene segments of lipid hydrocarbon chains. Chain unsaturation has only a minor influence on the ethanol distribution function. In all cases, the ethanol concentration in the bilayer core is significantly lower. At ambient temperature, all lipid-ethanol crosspeaks are positive. Crosspeak intensity decreases with increasing water content and increasing temperature, most likely, because of shorter correlation times of lipid and ethanol reorientation. This suggests a lifetime for specific lipid-ethanol contacts of about 1 ns. Lipid-ethanol and lipid-lipid crosspeaks reflect the high degree of motional disorder of lipids and incorporated ethanol in membranes and the rather arbitrary nature of the location of the lipid-water interface. The interface location of ethanol lowers interfacial energy of bilayers, increasing the area per lipid molecule. This provides more space for movement of the lipid hydrocarbon chains which become progressively more disordered with increasing ethanol concentration. Ethanol-induced chain disordering is smaller in polyunsaturated bilayers, most likely, because polyunsaturated hydrocarbon chains already occupy a larger area per molecule. The ethanol molecules at the lipid-water interface block pathways for water diffusion through lipid bilayers as seen in decreased rates of water permeation.

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INTRAMURAL RESEARCH PROJECT ZO1 AA 00099-02 LMBB
October 1, 1996 to September 30, 1997

Title of Project: Ion Gradients and Metabolic Energy in Animal Tissue

Principal Investigator: R. Veech, M.D., Ph.D. (Senior Investigator)
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Other Personnel: Y. Kashiwaya, Ph.D., LMBB
M.T. King, LMBB

Collaborating Units: Dept Biochemistry, Oxford University (Prof. G.K. Raddad; K. Clarke, Ph.D.); Dept Biochemistry and Biophysics, University of Pennsylvania (Prof. B. Chance; C. Keon)

Staff-Years: 2.5

Sample Type: Neither human subjects nor tissues

Summary of Work: During FY97 our work exploring the extent to which thermodynamics and kinetics can describe the control of flux in complex systems including glycolysis (J Biol Chem 1994;269:25502-14) and its interaction with the tricarboxcyclic acid (TCA) cycle, electron transport and oxidative phosphorylation (FASEB J 1995;9:651-8) continued. Most particularly, these studies concentrated on the limited extent to which alterations in a so-called "rate-limiting" enzyme can alter the overall flux in a metabolic pathway. Rather, the control of flux, and with it the control of phenotypic expression, is a result of the kinetic and thermodynamic parameters of each component in the system. It follows from such an analysis that the actions of drugs or hormones cannot be understood simply in terms of changes in one or a combination of "signally pathways." An example of how the complex hormonal signally pathways, initiated by binding of insulin to its receptor, may be duplicated by metabolic substrates was developed (Am J Cardiol 1997;80:50A-64A). Substrate overload is, of course, particularly relevant to alcoholism, where much of the toxicity results from the presence of abnormal levels of normal metabolic substrates.

In the coming year, the role of substrate depletion in TCA cycle of brain during alcohol withdrawal will be explored with the aim of finding alternative, non-addictive treatments.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00235-15 LMBB
October 1, 1996 to September 30, 1997

Title of Project: Nutritional Effects on Essential Fatty Acid Composition

Principal Investigator: N. Salem, Jr., Ph.D. (Senior Investigator)
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Staff-Years: 5.75

Sample Type: Human subjects (Minors & Interviews) and tissues

Summary of Work: Our studies have demonstrated that alcohol abuse leads to a decrease in the level of long chain polyunsaturated fatty acids like arachidonate (20:4n6) and docosahexaenoate (22:6n3). For example, there is a selective decrease in the level of 22:6n3 in the livers of rhesus monkeys, given alcohol on an ad libitum basis, that was associated with the development of liver fibrosis after three years. There is also a loss of docosahexaenoate (DHA) in the brains of cats and rhesus monkeys after chronic alcohol exposure. It is hypothesized that the lowered level of these important cell membrane constituents leads to alterations in cellular function that may underlie some aspects of alcohol-induced organ injuries and that prevention or restoration of this decrement in essential fatty acids (EFA) may be of therapeutic benefit to alcoholics. Progress has been made in understanding the underlying actions of alcohol on EFA metabolism. In studies of cats and rhesus monkeys exposed to chronic alcohol, there was a large increase in markers of lipid peroxidation. However, an increase in the enrichment of long chain polyunsaturates, especially DHA, with deuterium supplied by the 18-carbon fatty acid precursors indicated that EFA formation and accretion *in vivo* was increased. This contention is opposite to the commonly held notion that alcohol inhibits desaturase enzymes. These contentions were confirmed in a human study in which it was demonstrated that alcoholics accumulate greater amounts of deuterium-labeled DHA from d5-linolenic acid. These studies support the view that preformed 22:6n3 is essential for proper brain and liver function and that an important mechanism by which alcohol exerts adverse effects is through the antagonism of this fatty acid.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00262-13 LMBB
October 1, 1996 to September 30, 1997

Title of Project: Desaturation of Essential Fatty Acids Using Stable Isotope GC-MS

Principal Investigator: R. Pawlosky, Ph.D. (Research Fellow)
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Other Personnel: N. Salem, Jr., Ph.D., LMBB

Collaborating Units: None

Staff-Years: 1.1

Sample Type: Neither human subjects nor tissues

Summary of Work: Prolonged ethanol consumption has been found to lower the levels of polyunsaturated fatty acids especially, 20:4n6 (arachidonic acid) and 22:6n3 (docosahexaenoic acid), in plasma lipoproteins, erythrocytes, livers, brains and the retinas of primates and felines maintained on low essential fatty acid (EFA) diets (in the CNS, 22:6n3 is the principal fatty acid which decreased). The fatty acids, 20:4n6 and 22:6n3, are produced from the EFA, linoleate (18:2n6) and linolenate (18:3n3), respectively. A stable isotope gas chromatography mass spectrometry (GC/MS) method is being used to examine the effects of ethanol on the production of polyunsaturated fatty acids *in vivo*. Since lipid peroxidation may play a particular role in the depletion of polyunsaturated fatty acids, the levels of 4-hydroxy-alkenals and 8-isoprostane-F(2)-α in tissues are also being monitored using GC/MS procedures. In conjunction with metabolism studies, neural function at the level of the retina is being studied using electroretinography. Primates that have been consuming ethanol (on average 24% of energy) daily, for five years, have developed significant alcohol-induced liver pathology, including pericellular and venular fibrosis. The levels of 20:4n6 and 22:6n3 were significantly lower in plasma lipoproteins, erythrocytes and liver phospholipids in alcohol-exposed primates. In the brains, there were lower levels of 22:6n3 and a compensatory increase in 22:5n6 levels. The uptake of deuterium-labeled 18:3n3 or 18:2n6 into the plasma was about the same in both groups, however, a greatly increased level of lipid peroxidation as evidenced by higher levels of 4-hydroxynonenal and 8-isoprostane-F(2)-α in the plasma of the alcohol-exposed animals may be responsible for the loss of tissue poly-unsaturates. Our initial report, demonstrating alcohol-induced liver disease in primates, has been accepted for publication and is now in press. The above parameters will continue to be monitored for five-year periods in both groups of primates.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00072-06 LMBB
October 1, 1996 to September 30, 1997

Title of Project: Fluorescence Studies of Biophysical Properties of Polyunsaturated Phospholipids

Principal Investigator: B.J. Litman, Ph.D. (Senior Investigator)
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Other Personnel: K. Hines, LMBB
D. Mitchell, Ph.D., LMBB
C. Niebylski, Ph.D., LMBB

Collaborating Units: Laboratory of Chemical Physics, NIDDK, NIH (I. Levin, Ph.D.)

Staff-Years: 2.25

Sample Type: Neither human subjects nor tissues

Summary of Work: Neuronal and retinal tissue are high in phospholipids containing either one or two polyunsaturated acyl chains. Our studies have led to the development of a novel model for phospholipid acyl chain packing, which involves the formation of dynamic lateral domains in the surface of the membrane. Differential scanning calorimetry (DSC) of mixtures of a disaturated (di16:0) phosphatidylcholine (PC) and a dipolyunsaturated (di22:6n3) PC were studied. These lipids show poor miscibility in the liquid crystalline (LC) phase and demonstrate lateral phase separation in the gel phase. Fluorescence lifetime and anisotropy measurements, using the probe diphenylhexatriene (DPH), also indicates the presence of lateral heterogeneity in the membrane surface in both the gel and liquid crystal states. These studies support the presence of clusters or domains in mixtures of mixed chain and dipolyunsaturated phospholipids, as exist in both synaptosomal and retinal membranes. The propensity to form domains increases with increasing levels of acyl chain unsaturation, with 22:6n3 acyl showing the greatest level of domain formation. Both DSC and fluorescence data demonstrate that cholesterol preferentially interacts with saturated acyl chains and would, therefore, tend to concentrate in the more saturated regions of the membrane where it would effect both the size and lifetime of the domains. These findings have potentially important implications for integral membrane protein function. Domain formation would create regions of the membrane rich in highly unsaturated acyl chains. Proteins located in these regions would have different functional properties than those in more saturated regions of the membrane. Many neurotransmitter receptors and visual pigments are members of the G protein coupled-receptor family and reside in membranes containing very high levels of polyunsaturated acyl chains where domains of the type described are likely to occur. DSC measurements indicate that polyunsaturated phospholipids are more sensitive to the perturbing nature of ethanol. Therefore, the formation of highly unsaturated domains could result in regions of the synaptosomal membranes which are particularly sensitive to alcohols and other membrane soluble agents.

Fluorescent probes have been widely used to characterize the acyl chain packing properties of lipid bilayers in both model and biological membranes. We have extended the analysis of the time-resolved anisotropy decay of one of the most commonly used probes, DPH, to allow a measure of the acyl chain packing order in the membrane interior vs the portion of the chain closer to the interfacial region. These data complement the information from NMR and characterize the time averaged properties of the ensemble of molecules making up the bilayer. This analysis allows a more refined discrimination of the packing properties of phospholipid acyl chains and their interaction with cholesterol.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00080-04 LMBB
October 1, 1996 to September 30, 1997

Title of Project: The Influence of Protein-Lipid Interactions on Signal Transduction

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Collaborating Units: Laboratory of Chemical Physics, NIDDK, NIH (I. Levin, Ph.D.)

Staff-Years: 2

Sample Type: Neither human subjects nor tissues

Summary of Work: G protein-coupled receptors are ubiquitous components of signal transduction systems. This project is designed to assess the role of polyunsaturated phospholipids in modulating G protein-coupled signal transduction and to elucidate the mechanism of action of ethanol in these systems. The visual transduction pathway in the rod photoreceptor, a prototypical G protein-coupled system, is being used as a model system. The effect of alcohols and lipid composition on the kinetics and extent of formation of metarhodopsin II (MII), the G protein activating form of rhodopsin; MII/G protein complex formation; the rate of G protein activation; cGMP phosphodiesterase activation; and the GTPase activity of the G protein are being studied. Along with the functional measures in the transduction pathway, acyl chain packing properties of the phospholipid bilayer are being determined by use of time-resolved fluorescence spectroscopy. The isolation and reconstitution of rhodopsin into bilayers of defined lipid composition has allowed an evaluation of the role of phospholipid acyl chain composition. Short chain alcohols, such as ethanol, promote MII formation, while longer chain alcohols, such as decanol, are inhibitory. Intermediate length alcohols show a smooth transition from excitatory to inhibitory. Phospholipid bilayers containing 22:6n3 acyl chains are more sensitive to the effect of alcohols than are more saturated acyl chain phospholipids. The effects of ethanol, acyl chain composition and cholesterol are well correlated with changes in phospholipid acyl chain packing free volume, as characterized by the time-resolved fluorescence anisotropy behavior of the membrane probe, diphenylhexatriene (DPH). These results demonstrate that 22:6n3 containing phosphoplids are the best promoters of rhodopsin activation and provide unique structural features to the bilayer in the form of lateral domain formation. In addition, bilayers rich in 22:6n3 are least effected by the acyl chain ordering effects of cholesterol, which are found to be inhibitory with respect to rhodopsin activation.

The studies described here strongly support a lipid-mediated component in the mechanism of action for alcohols in modulating the activation of a G protein-coupled receptor. Our observations are best explained by a novel phospholipid acyl chain packing model, developed in this laboratory, in which the presence of phospholipids like those found in the retina and synaptosomes (e.g., polyunsaturated acyl chains, in mixed saturated-unsaturated and dipolyunsaturated acyl chain phospholipids) leads to the formation of lateral domains or clusters in the surface of the membrane. It would appear that these structural features play an important role in mediating receptor function in membranes containing long chain polyunsaturated phospholipids.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00089-03 LMBB
October 1, 1996 to September 30, 1997

Title of Project: Measurements and Metabolism of Neurosteroids in the Central Nervous System

Principal Investigator: H-Y. Kim, Ph.D. (Senior Investigator)
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Other Personnel: H.Z. Zhang, Ph.D., LMBB
B. Nardini, LMBB
M. Linnoila, M.D., Ph.D., LCS

Collaborating Units: None

Staff-Years: 0.7

Sample Type: Neither human subjects nor tissues

Summary of Work: The principal objective of this study is to determine the effect of ethanol on the metabolism of neurosteroids in the CNS. Neurosteroids such as 5α-pregnane-3α-ol-20-one (THP or Allopregnanolone) and 5a-pregnane-3α,21-diol-20-one (THDOC) have been shown to modulate the GABA/benzodiazepine binding sites and to exert anxiolytic and hypnotic effects. Modulation of these neurosteroids in the CNS by ethanol may be one of the under-lying mechanisms for stress-related situations in humans, a side effect often observed in alcoholics during withdrawal. As part of a continuing effort to establish a MS technique which has adequate sensitivity to routinely measure trace levels of neurosteroids in cerebrospinal fluid (CSF), we explored other approaches using gas chromatography/mass spectrometry (GC/MS) after derivatizing neurosteroids with an electron capturing moiety. In this mode, with the exceptions of progesterone and dihydroprogesterone, various neurosteroids were detected at 1 pg level. Using this method, characterization of the level of neuroactive steroids in rat brain and plasma as well as in monkey CSF is now in progress.

Title of Project: Alterations in Lipid Metabolism in the Nervous System by Ethanol

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Collaborating Units: None

Staff-Years: 4

Sample Type: Neither human subjects nor tissues

Summary of Work: The principal objective of this study is to elucidate metabolic and biological functions of polyunsaturated fatty acids, docosahexaenoic acid (22:6n3) and arachidonic acid (20:4n6) in the nervous system with particular reference to their modulation by ethanol. The effect of polyunsaturates on the survival of neuronal cells was investigated along with their effect on accumulation of phosphatidylserine (PS), which is thought to be involved in growth factor signaling leading to cell survival. We also found that polyunsaturated fatty acids affected the pineal function, since melatonin synthesis was significantly decreased with n3 deficiency. Polyunsaturated fatty acids, 20:4n6 and 22:6n3, prevented the apoptotic cell death of both Neuro 2A and PC-12 cells, assessed by DNA fragmentation and Hoechst staining. After 4 hours of exposure, only arachidonic acid (up to 25 mM) protected these cells from apoptosis induced by serum deprivation. However, 22:6n3 exerted its protective effect only after enrichment for at least 24 hours. While 22:6n3 was initially incorporated into triglycerides after 4 hours of exposure, a prolonged incubation led to the accumulation of this fatty acid in the aminophospholipids, phosphatidylethanol-amine (PE) and PS. Our data suggest that 22:6n3, as a membrane phospholipid constituent, especially in aminophospholipids, may be important for the protective effect. Even the enrichment 18:1n9 did not have any protective effect. The effect of polyunsaturated fatty acids on the accumulation of 22:6n3 in PS and modulation of PS biosynthesis by 22:6n3 was investigated using both animal and cell models, and the phospholipid molecular species were analyzed by electrospray liquid chromatography/mass spectrometry (LC/MS). The induction of n3 fatty acid deficiency significantly decreased the total polyunsaturated PS without affecting the total amount of polyunsaturated phospholipids in brain microsomes. The decrease in PS was mainly reflected by the incomplete replacement of 18:0:22:6 with 18:0:22:5 species as well as a decline in 18:0:20:4 PS. This reduction of PS was also accompanied by the accumulation of 18:0:22:5 PC, suggesting that the serine base exchange reaction using PC as a substrate may prefer 18:0:22:6 PC in comparison to 18:0:22:5 PC. The serine base exchange activity appeared to be affected by altering 22:6n3 composition in microsomal membranes, since the 18:0:20:4 PS also decreased in the microsomes from n3 deficient rats without altering the levels of potential substrates in PC and PE. In addition, PS biosynthesis assessed by [3H]serine incorporation decreased significantly after as little as a 30-minute incubation, suggesting that the presence of 22:6n3 species in membrane phospholipids may be important for the serine base exchange reaction, either as substrate molecules or as an enhancer of enzymatic activity. Similarly, C6 glioma cells cultured for 24 hours in docosahexaenoic acid-supplemented media (10-40 mM) significantly increased the synthesis of [3H]PS when compared with unsupplemented or 20:4n6-supplemented cells.

Our data show that neuronal and glial PS synthesis is sensitive to changes in the docosahexaenoate levels of phospholipids and suggest that 22:6n3 may be a modulator of PS synthesis. Polyunsaturates, especially 22:6n3, promote the accumulation of PS, in contrast to chronic ethanol treatment, which was found to significantly decrease the level of polyunsaturated PS without altering total polyunsaturate content in phospholipids.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00003-05 LMBB
October 1, 1996 to September 30, 1997

Title of Project: NMR Investigations of Cell Membrane Structure

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Staff-Years: 5.0

Sample Type: Neither human subjects nor tissues

Summary of Work: The objectives of this project are to: (1) investigate the interaction of alcohol with proteins and lipids in biological membranes; (2) study structure and dynamics of membranes composed of lipids with polyunsaturated fatty acids, such as docosahexaenoic acid (DHA) 22:6n3; and (3) study lipid-protein interactions related to alcoholism and lipid polyunsaturation.

The interface location of ethanol lowers interfacial energy of bilayers, increasing the area per lipid molecule. This provides more space for movement of the lipid hydrocarbon chains which become progressively more disordered with increasing ethanol concentration. Ethanol-induced chain disordering is smaller in polyunsaturated bilayers, most likely, because polyunsaturated hydrocarbon chains already occupy a larger area per molecule and are, therefore, less sensitive to ethanol-induced disordering. The ethanol molecules at the lipid-water interface block pathways for water diffusion through lipid bilayers as seen in decreased rates of water permeation.

Dehydration of membranes under polyethylene glycol (PEG)-controlled osmotic stress is equivalent to applying a lateral tension which compresses membranes. We have measured with 2H NMR the changes in lipid chain order resulting from stepwise osmotic dehydration and, on the same samples, with X-ray diffraction, the reduction of lamellar repeat spacing. The combination of NMR and X-ray methods allows exact determination of lipid area and lateral compressibility coefficients. Mixed-chain 18:0-22:6 PC has lower lateral compressibility coefficients than monounsaturated 18:0-18:1 PC. In the polyunsaturated mixed-chain 18:0-22:6 PC, the saturated 18:0 chain appears to be far less compressible than the polyunsaturated 22:6 chain.

The peptide fragment 828 848 with the sequence RVIEVVQGACRAIRHIPRRIR, from the carboxy terminal region of the envelope glycoprotein gp41 of HIV-1, deeply incorporates into negatively charged dimyristoylphosphatidylglycerol (DMPG) bilayers as an amphipathic alpha helix. Measurements on peptide with specifically deuterated isoleucine amino acids demonstrate that three of the four isoleucine residues are deeply imbedded in the hydrocarbon core of the membrane. The positively charged carboxy terminus of the peptide is located at the bilayer surface and shows a higher degree of flexibility.

FY 1997 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1996 - 30 SEPTEMBER 1997)

LABORATORY OF MOLECULAR & CELLULAR NEUROBIOLOGY

FORREST F. WEIGHT, M.D., CHIEF

DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
NATIONAL INSTITUTES OF HEALTH

SYNOPSIS

LABORATORY OF MOLECULAR & CELLULAR NEUROBIOLOGY

INTRODUCTION

In recent years, great progress has been made in understanding the function of the CNS at the cellular and molecular level. The LMCN was established, in FY 1992, to utilize this increased knowledge of neurobiology to investigate the cellular and molecular basis of alcoholism and alcohol abuse. The investigations in the Laboratory are directed toward elucidating the cellular and molecular mechanisms of alcohol's acute and chronic actions, such as intoxication, tolerance and dependence, as well as pathophysiologic phenomena, such as the neurotoxicity of alcohol, that results in cerebral atrophy and alcohol-induced alterations in neural development that are manifested as fetal alcohol syndrome (FAS).

Administratively, the Laboratory currently has two active sections: the Section on Physiology and the Section on Molecular Neuroscience. Dr. Forrest Weight is Chief, Section on Physiology, and Acting Chief, Section on Molecular Neuroscience. Initially the program had 19 FTE positions, but because of downsizing over the last four years, at the end of FY 1997 there were only five investigators on FTE positions. In addition, as the result of time limitations on non-tenured positions, four of those appointments will be terminated in the next year. A tenure-track cellular neurophysiologist/neuropharmacologist is currently being recruited, and recruitment of a tenure-track molecular neurobiologist is planned when sufficient space is available.

The Section on Physiology investigated the neuronal actions of alcohol and provided further evidence that neurotransmitter receptors are cellular sites of alcohol action in the nervous system. This evidence is an important advance in alcohol research, as it permits detailed investigation of the cellular and molecular mechanisms of alcohol actions in the nervous system. Moreover, since neurotransmitter receptors mediate synaptic communication between neurons, the demonstration that alcohol can affect the function of these receptors suggests that these actions may underlie many of the behavioral effects of alcohol.

The Section on Molecular Neuroscience used molecular biological approaches to investigate the molecular basis of alcohol action on neurotransmitter receptors. Studies of the Section have provided evidence that ethanol sensitivity of some types of neurotransmitter receptors is determined by the molecular structure of the receptor. In addition, studies are in progress on the molecular regulation of alcohol sensitivity. These studies hold the promise that such molecular biological approaches will advance our knowledge of the molecular basis of alcohol actions in the nervous system.

SECTION ON PHYSIOLOGY

The research program of the Section on Physiology is directed toward elucidating the cellular mechanisms of alcohol actions in the nervous system. The behavioral effects of alcohol are well known; however, the mechanisms by which alcohol produces those effects have not been established. The investigations in the Section use primarily electrophysiological methods, such as voltage-clamp techniques, to study the actions of alcohol on neuronal membrane receptors and ion channels.

Experiments on neuronal voltage-gated ion channels have indicated that in most cases these channels, which underlie the intrinsic electrical excitability of neurons, are relatively insensitive to pharmacologic concentrations of ethanol (5-100 μM). By contrast, a number of neuronal neurotransmitter-gated ion channels have been found to be sensitive to ethanol in pharmacologic concentrations. This type of neurotransmitter receptor mediates fast

excitatory or inhibitory synaptic transmission between neurons in the CNS. The effects of ethanol on these ion channels are discussed in more detail below.

Excitatory Amino Acid-Activated Channels: Glutamate is the major excitatory neurotransmitter in the mammalian CNS. Glutamate activates at least three types of neurotransmitter-gated ion channels, designated by their responses to the agonists N-methyl-D-aspartate (NMDA), kainate and quisqualate (or AMPA).

NMDA-gated channels mediate a slow component of synaptic excitation and are thought to be involved in several types of important neural phenomena including: cognitive function, motor control, synaptic plasticity and certain types of learning. In hippocampal neurons, ethanol has been found to inhibit NMDA-activated current in a concentration-dependent manner over the concentration range 5-50 μM, a range that produces intoxication. The potency of several short chain alcohols for inhibiting NMDA-activated current is related to their intoxicating potency, suggesting that alcohol-induced inhibition of NMDA channel function may contribute to the neural and cognitive impairments associated with intoxication. Investigations on the mechanism involved in the inhibition of NMDA current by ethanol indicate that ethanol does not inhibit NMDA current by block of the ion channel, by altering the ion selectivity of the channel or by interaction with several regulatory sites on the receptor-channel. Single channel experiments suggest that ethanol inhibits NMDA-activated current by altering channel gating. Inhibition of NMDA receptor-mediated responses by a series of straight chain alcohols exhibits a distinct cutoff for alcohols with nine or more carbon atoms. This cutoff is similar to the cutoffs reported previously for two behavioral indices of intoxication, ataxia and loss-of-righting reflex, suggesting that alcohol inhibition of NMDA receptors may contribute to the behavioral manifestations of alcohol intoxication.

Kainate- and quisqualate-activated channels (non-NMDA glutamate receptor-channels) mediate fast transmission at the majority of excitatory synapses in the CNS. In concentrations <50 μM, ethanol has relatively little effect on the responses of these non-NMDA glutamate receptor-channels. In concentrations >50 μM, however, ethanol produces an increasing concentration-dependent inhibition of these currents, with 200 μM ethanol inhibiting by about 45%. Blood-ethanol concentrations >50 μM are associated with signs of general anesthesia. Since this is the concentration range that shows increasing inhibition of kainate- and quisqualate-activated responses by ethanol, and these channels mediate fast synaptic transmission at most excitatory synapses in the CNS, it seems possible that the ethanol inhibition of kainate- and quisqualate-receptor function may contribute to the general anesthetic effects of ethanol. This hypothesis is supported by the observations that the general anesthetic agents, trichloroethanol (the active metabolite of chloral hydrate), barbiturates and volatile general anesthetics, all inhibit kainate- and quisqualate-activated currents in an anesthetic concentration range.

Inhibitory Amino Acid-Activated Channels: The major inhibitory neurotransmitter in the brain is γ-aminobutyric acid (GABA). Ethanol has been reported to have different effects on GABA-gated ion channels ($GABA_A$ receptors) in different preparations. In cultured mouse cortical and hippocampal neurons, 1-40 μM ethanol had been found to produce a concentration-dependent potentiation of GABA-activated current in some, but not all, neurons tested. By contrast, ethanol concentrations from 10-100 μM have no significant effect on GABA-activated current in freshly isolated adult rat dorsal root ganglion (DRG) neurons. One hypothesis that has been proposed to explain why only some $GABA_A$ receptors are sensitive to ethanol is that the receptors must be phosphorylated by protein kinase C (PKC). However, our experiments attempting to induce ethanol sensitivity of $GABA_A$ receptors by procedures that would activate PKC have been unsuccessful. On the other hand, straight-chain alcohols with three or more carbon atoms consistently potentiate GABA-activated current with increasing potency as the carbon chain-length is increased up to 12 carbon atoms. However, maximally attainable concentrations of alcohols with 12 or more carbon atoms do not enhance GABA-activated current. This cutoff for alcohol potentiation of GABA-activated current differs from the cutoffs reported previously for behavioral measures of alcohol intoxication, but is similar to the cutoff reported for anesthesia in tadpoles.

Serotonin-Activated Channels: Serotonin type 3 ($5HT_3$) receptors have been implicated in alcohol abuse and alcoholism. In NCB-20 cells and nodose neurons, ethanol potentiates

$5HT_3$ receptor-mediated current in a concentration-dependent manner over the concentration range 25-100 μM, at low agonist concentrations. A potentiation of 59% is observed with an ethanol concentration of 100 μM. Potentiation by ethanol decreases with increasing serotonin concentration, suggesting that ethanol may increase the potency of serotonin action. Potentiation of $5HT_3$ receptor-mediated current by a series of straight-chain alcohols exhibits a distinct cutoff for alcohols with ≥6 carbon atoms.

ATP-Activated Channels: Adenosine 5'-triphosphate (ATP) has been demonstrated to function, extracellularly, as an excitatory neurotransmitter and to activate ligand-gated ion channels in both the central and peripheral nervous system. There appear to be several types of ATP-gated ion channels in mammalian neurons, based on activation and desensitization kinetics and pharmacologic sensitivity. Ethanol inhibits the function of one type of ATP-gated channel over a pharmacologic concentration range, with an IC_{50} value of 68 μM. Methanol is less potent and 1-propanol is more potent in inhibiting the ATP-activated current; however, 1-butanol and 1-pentanol are without effect on this current. In addition, the intracellular application of 100 μM ethanol does not affect the inhibition by extracellularly applied ethanol, the inhibition is not affected by the intracellular application of activators and inhibitors of G-proteins, and the inhibition is not affected by activators and inhibitors of protein kinase A (PKA) or PKC. The mechanism of ethanol inhibition of ATP-gated channels differs from the mechanism of ethanol inhibition of NMDA receptors. For NMDA receptors, ethanol decreases E_{max} of the agonist concentration-response curve without affecting the EC_{50}, whereas for the ATP-gated channels, ethanol shifts the ATP concentration-response curve to the right in a parallel manner, increasing the EC_{50} for ATP without altering its maximal response. The effect of ethanol on the function of ATP-gated channels appears to be a competitive type of inhibition; however, an ethanol-induced decrease in the affinity of the agonist binding site would also result in this type of inhibition. To distinguish between these mechanisms, the effect of ethanol was studied on the activation and deactivation rates of ATP-activated current. Ethanol decreased the time constant of deactivation of ATP-activated current, without affecting the time constant of activation. The observations are not consistent with a competitive mechanism of inhibition, but are consistent with an allosteric action of ethanol to decrease agonist affinity.

Nicotinic Acetylcholine Channels: Although the nicotinic acetylcholine (nACh) receptor-channels are the most extensively characterized neurotransmitter-gated ion channels in terms of molecular biology, the effects of ethanol on the function of these receptors have not been well characterized with modern electrophysiological techniques. In studies on mouse αβεδ nACh receptors, ethanol concentrations from 10-150 μM produce a concentration-dependent potentiation of currents activated by low concentrations of ACh. Associated with the ethanol-induced potentiation is an increased desensitization rate of the current. However, with ACh concentrations >25 μM, ethanol reduces peak current amplitude, presumably due to the rapid onset of desensitization, as occurs with high agonist concentrations.

Summary and Conclusions: The studies by the Section on Physiology indicate that ethanol can affect the function of a number of different types of neurotransmitter-gated ion channels. Thus, these receptors are membrane proteins that are involved in neuronal excitability that are sensitive to the action of ethanol. These studies also show that a series of straight-chain alcohols exhibit a potency cutoff for affecting the function of all of the neurotransmitter-gated ion channels tested. These potency cutoffs are consistent with the hypothesis that alcohols affect the function of these receptors by interacting with a hydrophobic pocket of circumscribed dimensions on the receptor protein. The observation, that the cutoff is different for each receptor type studied, suggests that the size of the hydrophobic pocket is different on different types of receptors. The studies also show that alcohol effects on the function of different types of neurotransmitter-gated ion channels can involve different types of specific mechanisms. The studies in the Section of Physiology suggest that the application of electrophysiological approaches to the study of alcohol actions on neurons will advance our knowledge of the cellular basis of alcohol actions in the nervous system.

SECTION ON MOLECULAR NEUROSCIENCE

The research activities of the Section on Molecular Neuroscience are directed toward understanding actions of alcohol in the nervous system at the molecular level. The Section uses a combination of molecular biological and electrophysiological research methods to address these questions. In the studies carried out by the Section, the effects of ethanol have been studied on the physiology and pharmacology of recombinant neurotransmitter receptors using *Xenopus* oocytes or various cell lines as expression systems. Those studies are summarized briefly below.

Recombinant NMDA Receptors: Although ethanol inhibits NMDA receptor-mediated responses in a number of regions of the nervous system, the sensitivity of NMDA receptors to ethanol is different in different brain regions and in different types of neurons. Cloning studies have revealed a molecular diversity of NMDA receptors and *in situ* hybridization indicates a differential distribution of different subunits throughout the brain, raising the question of whether differences in NMDA receptor subunit composition might be responsible for the differences in NMDA receptor sensitivity to ethanol in different types of neurons.

The ethanol sensitivity of different NMDA receptor subunits was studied using recombinant NMDA receptor subunits expressed in *Xenopus* oocytes. Various NMDA receptor subunit combinations were found to exhibit a differential sensitivity to ethanol. The heteromeric subunit combinations $\varepsilon1/\zeta1$ and $\varepsilon2/\zeta1$ are significantly inhibited by 50 μM ethanol, whereas the heteromeric combination $\varepsilon3/\zeta1$ and the homomeric $\zeta1$ subunits are not significantly affected by this ethanol concentration. The sensitivity of the $\varepsilon4/\zeta1$ subunits is similar to that of the $\varepsilon3/\zeta1$ subunits. In addition, there are differences in the ethanol concentration-response curves for different subunit combinations. The observations are consistent with the idea that NMDA receptor subunit composition may contribute to differences in ethanol sensitivity observed in different brain regions and in different types of neurons.

Recombinant Non-NMDA Glutamate Receptors: The ethanol sensitivity of non-NMDA glutamate receptors was studied using the recombinant GluR1-3 subunits expressed in *Xenopus* oocytes. Ethanol consistently inhibits kainate-activated current of these receptors in a concentration-dependent manner over the concentration range 10-500 μM. The ethanol inhibition of the responses of the GluR1+2+3 heteromeric combination has an IC_{50} value of 176 μM, and the IC_{50} value for inhibition of the GluR3 subunit is 212 μM. These values are in a similar range to the ethanol sensitivity we found for non-NMDA glutamate receptors on hippocampal neurons. In addition, for a series of straight-chain alcohols from methanol to heptanol, the potency for inhibition of GluR receptor-mediated responses increases in proportion to the chain-length or hydrophobicity of the alcohol. However, despite increased hydrophobicity, a distinct cutoff for inhibition of these receptors is observed for alcohols with ≥8 carbon atoms for GluR1, ≥9 carbon atoms for GluR3, and ≥10 carbon atoms for non-NMDA glutamate receptors on hippocampal neurons.

Recombinant and Expressed $GABA_A$ Receptors: It has been suggested that the ethanol sensitivity of $GABA_A$ receptors requires the presence of the $\gamma2L$ subunit of these receptors, based on studies in *Xenopus* oocytes expressing either long-sleep (LS) mouse brain mRNA or mouse $GABA_A$ $\alpha1\beta1\gamma2L$ subunit cRNA. However, despite several years of research attempting to repeat the observations on which this conclusion was based, we have been unable to find effects of ethanol on $GABA_A$ receptor-mediated responses in *Xenopus* oocytes expressing either LS mouse brain mRNA or mouse $GABA_A$ $\alpha1\beta1\gamma2L$ subunits. Our inability to confirm the observations on which the $\gamma2L$ hypothesis was based suggests that the molecular determinants of $GABA_A$ receptor sensitivity to ethanol is not a resolved issue.

Recombinant nACh Receptors: The α7 subtype of nACh receptors has recently been cloned and characterized. It is found in the mammalian CNS and it has been suggested that it may be involved in nicotine addiction. Little is known, however, about the effect of ethanol on this receptor. When we studied the effect of ethanol on recombinant $nACh_{\alpha7}$ receptors, we were surprised to find that ethanol inhibits the function of this receptor, because ethanol potentiates the function of muscle-type nACh receptors. The inhibition of $nACh_{\alpha7}$ receptors by ethanol is of the non-competitive type, which is similar to what we have observed for

ethanol inhibition of NMDA- and non-NMDA-glutamate receptor-mediated responses, but it differs from the response of ATP-gated channels, where ethanol appears to decrease the affinity of the agonist binding site. On the other hand, for the heteromeric αβεδ nACh receptors, the ε subunit appears to be required for ethanol action, because ethanol potentiation is not observed for nACh receptors composed of αβδ subunits.

Recombinant $5HT_3$ Receptors: As noted, studies in the Section on Physiology have shown that ethanol can potentiate $5HT_3$ receptor-mediated ion current in neurons and neural cell lines. However, it was also found that $5HT_3$ receptor-mediated current is insensitive to ethanol in approximately 15% to 25% of these cells. Since a $5HT_3$ receptor subunit has been cloned, we expressed this receptor in *Xenopus* oocytes to study the molecular determinants of ethanol sensitivity of these receptors. We found that for the recombinant homomeric $5HT_3$ receptor, ethanol potentiates the current activated by low concentrations of 5HT in all of the cells studied. Since this clone of the $5HT_3$ receptor has consensus sequences for phosphorylation, and PKC activation potentiates the response of these receptors, experiments are in progress, using molecular biological methods such as site-directed mutagenesis, to determine whether these consensus sequences for phosphorylation can regulate the sensitivity of $5HT_3$ receptors to ethanol.

Recombinant Chimeric $nACh_{\alpha 7}$-$5HT_3$ Receptors: Recombinant chimeric membrane proteins have been extremely valuable for determining relationships between functional properties and defined structural domains of these proteins. One such construct, a chimeric neurotransmitter receptor from two different neurotransmitter-gated ion channels, with the N-terminal domain from the $nACh_{\alpha 7}$ receptor and the transmembrane and C-terminal domains from the $5HT_3$ receptor, manifests activation by nicotinic agonists but the channel specificities of $5HT_3$ receptors. Since ethanol inhibits the response of $nACh_{\alpha 7}$ receptors and potentiates the response of $5HT_3$ receptors, we used the chimeric $nACh_{\alpha 7}$-$5HT_3$ receptor to study whether the modulatory actions of ethanol are associated with the N-terminal or the transmembrane and C-terminal domains of the receptor. Ethanol was found to inhibit the response of this chimeric receptor. Since this inhibition by ethanol is not significantly different from the inhibition of $nACh_{\alpha 7}$ receptors by ethanol, the observation suggests that the inhibition is mediated by the N-terminal domain of the receptor.

Summary and Conclusions: The studies of the Section on Molecular Neuroscience have provided evidence that the ethanol sensitivity of some types of neurotransmitter receptors is determined by the molecular structure of the receptor. In addition, studies are in progress on the molecular regulation of alcohol sensitivity of neurotransmitter receptors. These studies hold the promise that such molecular biological approaches will advance our knowledge of the molecular basis of alcohol actions in the nervous system.

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INTRAMURAL RESEARCH PROJECT ZO1 AA 00007-05 LMCN

October 1, 1996 to September 30, 1997

Title of Project: Molecular Neurobiology and Alcohol Actions

Principal Investigator: F.F. Weight, M.D. (Senior Investigator)
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Collaborating Units: Inst Pasteur, Paris (J.-L. Eisele, Ph.D.; J.-P. Changeux, M.D.); Centre Med U, Geneva (D. Bertrand, Ph.D.); RIKEN, Japan (M. Matsuzawa, Ph.D.); Johns Hopkins U (R. Potember, Ph.D.); NINDS, NIH (J. Harvey-White, Ph.D.)

Staff-Years: 3.5

Sample Type: Neither human subjects nor tissues

Summary of Work: The molecular basis of alcohol actions in the nervous system is poorly understood. This project studied the molecular structure-function relationships of neurotransmitter receptors and the molecular mechanisms of alcohol action on those receptors, using a combination of both molecular biological and electrophysiological techniques. The ethanol sensitivity of $GABA_A$ receptors was studied using $GABA_A$ receptors expressed in *Xenopus* oocytes. Responses of $GABA_A$ receptor subunit combination, $\alpha1\beta1\gamma2L$, were not affected by ethanol concentrations from 5-100 mM nor were the responses of $GABA_A$ receptors from long-sleep (LS) or short sleep (SS) mice, which does not confirm previous reports. The inhibition of non-NMDA glutamate receptors by a series of n-alcohols was studied in *Xenopus* oocytes expressing GluR1 and GluR3 receptors and compared to that in hippocampal neurons. Potency for inhibition increased in proportion to chain-length, but inhibition was not observed with 1-octanol for GluR1, 1-nonanol for GluR3 nor 1-decanol for the neurons. The observations are consistent with an interaction of the alcohols with a hydrophobic pocket on the receptor protein, suggesting that the size of the pocket may differ on different subunits of the receptor. The potentiation of nACh receptors by ethanol was investigated in HEK-293 cells expressing mouse subunits. In cells expressing $\alpha\beta\delta\gamma$ subunits, 10-150 μM ethanol potentiated current activated by low ACh concentrations. By contrast, in cells expressing $\alpha\beta\delta$ subunits, ethanol (10-100 μM) did not affect ACh-activated current. The observations suggest that the γ subunit is required for the ethanol action on these receptors. To localize ethanol's action, its effect was studied on $nACh_{\alpha7}$ and $5HT_3$ receptors and a chimeric $nACh_{\alpha7}$-$5HT_3$ receptor expressed in *Xenopus* oocytes. Ethanol (10-100 μM) inhibited $nACh_{\alpha7}$ receptors, potentiated $5HT_3$ receptors and inhibited the chimeric receptor in a manner identical to that of $nACh_{\alpha7}$ receptors. Since the N-terminal domain of the chimeric receptor was from the $nACh_{\alpha7}$ receptor, the observations suggest that ethanol action involves the N-terminal domain of the receptor.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00479-14 LMCN
October 1, 1996 to September 30, 1997

Title of Project: Synaptic Mechanisms and Alcohol Actions

Principal Investigator: F.F. Weight, M.D. (Senior Investigator)
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Bethesda MD 20892

Other Personnel: C. Li, M.D., LMCN
R. Peoples, Ph.D., LMCN
J. Wright, Ph.D., LMCN

Collaborating Units: None

Staff-Years: 2.5

Sample Type: Neither human subjects nor tissues

Summary of Work: The cellular basis of alcohol actions in the nervous system is poorly understood. Recent studies have shown that alcohols can affect the function of certain neurotransmitter receptors, however, the cellular mechanisms involved in those effects have not been established. This project studied the cellular mechanisms involved in the effects of alcohol on neurotransmitter receptor function using electrophysiological techniques. The inhibition on NMDA receptor-mediated responses by ethanol was investigated in mouse hippocampal neurons. In whole-cell patch-clamp experiments, ethanol decreased E_{max} of the agonist concentration-response curve, without affecting either the EC_{50} or the apparent Hill coefficient. The inhibition was voltage-independent and did not involve the glycine, polyamine, proton, rhedox, ketamine, Mg2+ or Zn2+ regulatory sites on the receptor. In single-channel experiments, ethanol inhibition of NMDA-activated current did not involve changes in fast, closed-state kinetics, changes of open channel conductance or block of the open channel. At the IC_{50} for ethanol inhibition, it is estimated that the open channel lifetime is decreased by 28% and the frequency of opening is decreased by 31%. The data are consistent with ethanol being an allosteric modulator of channel gating which reduces agonist efficacy. The potentiation of γ-aminobutyric acid type A ($GABA_A$) receptor-mediated responses by a series of primary alcohols was also studied in mouse hippocampal neurons. From 1-butanol to 1-undecanol, the potency of the alcohols for potentiating $GABA_A$ receptor-mediated current increased exponentially with increasing carbon chain length. However, the potency of 1-duodecanol for potentiating $GABA_A$ responses was not significantly different from that of 1-undecanol and alcohols with ≥12 carbon atoms (1-tridecanol and 1-tetradecanol) had no significant effect on $GABA_A$ receptor-mediated current. This cutoff in the potency of n-alcohols for potentiation of $GABA_A$ receptor-mediated responses is consistent with an interaction of the alcohols with a hydrophobic pocket on the receptor protein.

Title of Project: Nerve Cell Excitability and Alcohol Actions

Principal Investigator: F.F. Weight, M.D. (Senior Investigator)
LMCN, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: None

Collaborating Units: University of Pittsburgh School of Medicine (W.C. de Groat, Ph.D.; N. Yoshimura, Ph.D.)

Staff-Years: 0.1

Sample Type: Neither human subjects nor tissues

Summary of Work: Alcohol is classified pharmacologically as a CNS depressant. The cellular mechanisms that underlie this alcohol-induced depression of nervous system excitability, however, are poorly understood. This project investigated the intrinsic mechanisms involved in the regulation of nerve cell excitability and the effects of ethanol on those mechanisms.

Whole-cell patch-clamp recording in combination with axonal tracing techniques were used to examine the electrical properties of rat DRG neurons. The majority of neurons were small (70%) and expressed high-threshold tetrodotoxin (TTX)-resistant Na+ channels and slowly inactivating K+ channels (K-A). The large-sized neurons had low-threshold TTX-sensitive Na+ channels and fast inactivating K-A channels. Half-maximal conductances for activation of TTX-resistant and TTX-sensitive Na+ currents were -10.3 and -25.3 mV. TTX-resistant and TTX-sensitive Na+ currents were half-inactivated at -25.3 and -56 mV. In TTX-resistant neurons, transient K+ current (I-A) had half-maximal conductance at -40.8 mV, was half-inactivated at -77.5 mV and exhibited a slower decay constant (τ, 240 ms) than I-A current in TTX-sensitive neurons (τ, 20 ms). Na+ and K+ currents were similarly characterized in rat major pelvic ganglion (MPG) neurons. Na+ current was reversibly blocked by 1 μM TTX. Na+ conductance reached half-maximal activation at -21.5 mV, and was half-maximally inactivated at -57.5 mV. A fast-transient I-A current was half-maximally activated at -21.2 mV and half-maximally inactivated at -76.5 mV. A delayed K+ current was reduced 25-35% by the bath application of Cd2+ or 0 Ca2+; 4-AP (2 μM) suppressed I-A current by 75% and delayed K+ current by 60%; and TEA (10 μM) suppressed delayed K+ current by 90% and I-A current by 16%. The results indicate that MPG neurons have a TTX-sensitive Na+ current and three distinct types of K+ currents: I-A, Ca2+-activated and delayed rectifier. The effect of ethanol was tested on voltage-gated ion channels in several types of neurons and was found to have little or no effect on channel function in a pharmacologic concentration range (5-100 μM).

FY 1997 ANNUAL REPORT SUMMARIES

(1 OCTOBER 1996 - 30 SEPTEMBER 1997)

LABORATORY OF NEUROGENETICS

DAVID GOLDMAN, M.D., CHIEF

DIVISION OF
INTRAMURAL CLINICAL & BIOLOGICAL RESEARCH
NATIONAL INSTITUTE ON ALCOHOL ABUSE AND ALCOHOLISM
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SYNOPSIS

LABORATORY OF NEUROGENETICS

INTRODUCTION

The primary mission of this laboratory is to identify the vulnerability and protective alleles which underlie alcoholism's measured heritability. Identification of these alleles will lead to better understanding of mechanisms of vulnerability, molecular diagnostic markers to individualize treatment and gene-environment interactions. Two complementary approaches, whole genome linkage analysis and direct scanning of candidate genes, are being used to identify these alleles. These approaches have strengths and weaknesses that are reciprocal. Direct gene analysis is being used to exclude functionally-related families of neurotransmitter genes under models of high genetic heterogeneity. Paradoxically, with direct gene analysis, the likelihood of detecting predisposing alleles is enhanced by causal heterogeneity or polygenicity which are obstacles for detection of loci by whole genome linkage scan. For example, Risch has estimated that a locus accounting for less than 10% of the variance may be virtually undetectable by sib-pair linkage. However, for alcoholism, it is currently only practical to screen for sequence variation (known regions of the genome which are several kilobases in size). Conversely, linkage will detect unknown loci (albeit of larger effect size) wherever they are located. The direct gene analysis and whole genome linkage approaches are complementary because the same linkage datasets are useful for testing the effect on phenotype of candidate alleles identified by scanning and because the whole genome linkage studies, if successful, lead to direct gene analyses.

Because alcoholism is both common and etiologically complex, our linkage studies capitalize on the greater genetic and environmental homogeneity of American Indian and Finnish isolates. For the same reasons, we are using neurophysiologically and neurochemically defined traits which more closely reflect endophenotypes, for example, in studies of families with the low voltage α (LVA) EEG trait and those with low CSF 5HIAA probands. Alcohol sensitivity, personality traits and comorbid diagnosis are other subgroup-defining phenotypes available in all or certain datasets. These approaches serve to reduce genetic heterogeneity and to direct the molecular genetic studies which include studies of the functional significance of alleles.

The genetics of alcoholism also includes interindividual differences in sensitivity to alcoholism, including its deleterious consequences. Our research in this domain has been directed toward mechanisms of damage and damage prevention (P450, DNA repair) and toward understanding the control of expression of genes important in alcoholism vulnerability (genes such as tryptophan hydroxylase (TPH) and the serotonin transporter).

Familiality and genetic linkage of alcoholism in American Indians: One group of U.S. communities in which alcoholism and its devastating consequences are frequently pervasive is the American Indian population. For the development of treatment and prevention strategies, it is vital to establish the role and identity of causative factors in such populations, which also offer the advantage of greater genetic and environmental homogeneity than the U.S. population as a whole.

We identified moderate to strong evidence for two potential alcoholism-vulnerability genes in a Southwestern American Indian tribe with a high rate of alcoholism (85% of males, >50% of females). To accomplish this, a very large family (N=582) was systematically interviewed, evaluated for familial transmission of alcoholism and then tested, in collaboration with investigators from NIDDK (W.Knowler, R.Hanson, P. Bennett), for genetic linkage using a large (N=517) panel of DNA markers which covered all human chromosomes except the sex chromosomes: X and Y. Significant and progressively declining risk for alcoholism was seen in females who were at the 1st, 2nd and 3rd degrees of genetic relationship to a female alcoholic. This corresponds to a heritability (role of genotype in vulnerability) of about 40%,

similar to the general U.S. population. The same trend was present in males but, nonsignificant, perhaps due to the very high rate of alcoholism in this particular population.

The strongest evidence for an alcoholism-vulnerability gene was, at the chromosome 11p telomere, directly at the location of the dopamine DRD_4 gene that has been previously implicated in impulsive behavior. On a conservative basis, this statistical result could have been observed randomly in 1 in 6 whole genome scans, emphasizing the need for replication and extension of these results. A moderate to strong linkage signal was also found on chromosome 4p, at the location of a cluster of $GABA_A$ receptor genes. These results are convergent with extensive pharmacological evidence (for example, the cross tolerance between alcohol and drugs which act at these receptors) and some previous animal genetic evidence implicating $GABA_A$ receptors in alcoholism. Finally, there was moderate evidence from this whole autosome scan for linkage of alcohol dependence to the chromosome 4q region which contains the alcohol dehydrogenase genes. This result is convergent with the recently established role for variants of alcohol dehydrogenase genes in alcoholism risk.

Binge drinking is a common pattern of behavior in many American Indian communities but the significance of binge drinking is disputed; it is often argued that binge drinking is benign or even beneficial in Indians. However, in the Southwestern tribe we studied, almost all of the large fraction of the population who were binge drinkers were also alcoholics and binge drinkers tended to become alcoholic at a younger age. Furthermore, regardless of whether binge drinkers met criteria for alcoholism, they were dramatically worse off in the four symptom categories evaluated: social, work, violence/lawlessness and physical. It is of particular interest that binge drinking interfered with ability to function both at home and at work. Analysis of psychiatric comorbidity reveals the same heavy clusterings of psychiatric disorders with alcoholism as observed in the general population of the U.S., in studies such as the National Comorbidity Survey. These results may help to lay to rest the misconception that drinking, particularly binge drinking, whatever its origin, is other than deleterious to American Indian communities.

Current directions for linkage studies in American Indians are: 1) a linkage study in a low-alcoholism tribe, led by Dr. J. Long in collaboration with B. Albaugh (Center for Behavioral Studies); 2) an EEG linkage study in a tribe with a relatively high rate of alcoholism; and 3) the Ten-Tribe Study, a genetic epidemiological study comparing tribes with low and high rates of alcoholism to identify gene-environment interactions (in concert with M. Koss, U Arizona). These studies are partially supported with funds from the Minority Health Initiative and data collections are by peer-reviewed contracts. The EEG study will primarily involve neuropsychological assessment, of families we have previously studied, utilizing additional interviews and blood samples. The Ten-Tribe Study (N=3000) will investigate interaction of environment and genotype on differing rates of alcoholism as well as personal and community-wide effects of alcoholism, including trauma, in tribes with low and high rates of alcoholism. The differences in alcoholism rates between tribes are thought, on the basis of local health statistic data, to be dramatic, but have not been systematically documented. In the Ten-Tribe Study, 300 demographically-sampled subjects from each of ten tribes are being psychiatrically interviewed (AUDADIS) and a blood sample will be collected for DNA.

Identification of variants of candidate genes for alcoholism and other behaviors and linkage to behavior: Among neurotransmitters, serotonin is important for its roles in behavioral inhibition and anxiety. Serotonin is thought to have an involvement in diverse disorders including schizophrenia, autism and alcoholism. Dopamine, endogenous opioids and $GABA_A$ receptors are critical for drug-mediated reinforcement and for other behaviors. A key step toward progress in understanding the genetics of complex diseases, such as alcoholism, is the identification of functionally significant gene variants (alleles) which can alter the function of neurotransmitters such as these. We identified a substantial collection of such variants and are relating them to alcoholism and other behaviors.

With regard to serotonin, amino acid substitutions of the serotonin receptors, designated $5HT_{1A}$, $5HT_{2A}$ and $5HT_{2C}$, were discovered which alter receptor function. Each of the receptors is thought to be an important pharmacological and natural target of action in serotonin function and two of these variants are common in the general population of the United

States. The $5HT_{1A}$ is one of two autoregulatory serotonin receptors and $5HT_{2A}$ and $5HT_{2C}$ are possible sites of action of the atypical antipsychotic drug, clozapine. The $5HT_{2A}$ and $5HT_{2C}$ variants have already been related to clozapine response, however, there are also contradictory results on this question and more data will be required. Therefore, these functional variants may provide important clues to the origins of naturally occurring behavioral variation, including psychiatric disease.

Recently we have discovered common amino acid substitutions in two other receptors critical for understanding alcoholism. One of these is a common polymorphism (variant) of the μ opioid receptor, found by A. Bergen et al. This receptor is thought to be involved in both pain and reinforcement and is a major site of action of the drug, naltrexone, which is promising in the treatment of alcoholism. N. Iwata et al. have also found a common amino acid substitution of the $GABA_A$ α6 receptor, the same gene which, in a rodent model, confers susceptibility to alcohol sensitivity. Studies are underway to relate these genetic variants to human behavior.

LINKAGE OF CANDIDATE GENE VARIANTS TO BEHAVIOR

A critical test of the DRD_2 dopamine receptor, "Reward Deficiency Gene" hypothesis, was performed in a very large Southwestern American Indian linkage dataset. The linkage study was done with three DRD_2 markers, including an amino acid substitution, which dramatically impairs signal transduction by the activated receptor and which is far more abundant in this particular population as compared to Caucasians. There was neither genetic linkage nor genetic association to the dopamine receptor markers, including the functional amino acid substitution, suggesting that the DRD_2 "Reward Deficiency Gene" hypothesis may be laid to rest.

An association of a genetic variant of TPH to suicide was detected. TPH is rate-limiting for the synthesis of serotonin, which is involved in impulsive behavior. In studies led by D. Nielsen, we were able to replicate this association earlier this year, helping to solidify one of the first strong clues to the inherited biologic contribution to suicide, a scourge of society.

Others have linked a functional genetic variant (5HTTLPR) of the serotonin transporter gene, the target of action of serotonin reuptake inhibitors used in the treatment of anxiety and depression, to anxiety and neuroticism. In a large study on sib-pairs, collected at the University of Helsinki, C. Mazzanti et al. were able to replicate the linkage to anxiety. The HTTLPR variant may have diverse effects on behavior and, working with investigators at the NIMH, C.Mazzanti et al. have recently found strong indications that it is predisposing to seasonal affective disorder (SAD) and the clinical presentation of schizophrenics--specifically, their degree of psychoticism.

EEG PHENOTYPE/PHENOTYPE ASSOCIATIONS

Neuropsychological markers for alcoholism vulnerability include the P300 event-related potential, the LVA EEG trait, sensitivity to acutely administered alcohol, personality measurements and neurochemical levels. All may identify genetically and physiologically more distinct subgroups of alcoholics as well as particular individuals who are at greater risk for alcoholism. Several other markers (low platelet monoamine oxidase and platelet adenylate cyclase) are found in a large fraction of alcoholics. These are more likely to represent long-lasting state changes associated with alcoholism and, for this reason, were not selected as a focus for linkage studies.

The LVA EEG trait is an abundant, stable, neurophysiologic trait which appears to be transmitted in an autosomal dominant fashion. Earlier, M-A. Enoch reported a phenotype/ phenotype association between LVA and alcoholism and recently replicated this observation. The frequency of LVA is about four times higher in alcoholics as compared to the general population and alcoholics with anxiety disorders are still more likely to have LVA. A linkage study, by Dr. Urbanek et al., failed to confirm the chromosome 20q linkage for LVA detected by

Steinlein and colleagues. This result may be due to heterogeneity. Our LVA genome scan is incomplete (79 STRs, ultimately 386) and has thus far yielded no positive linkages. LVA is a relatively common trait and considerable genetic heterogeneity may be present, thus, reducing the power to detect linkage without large families. To generate large families individually capable of yielding significant linkage results, EEG studies have been extended to include American Indian families from a Plains tribe in Oklahoma from whose members we have psychiatric diagnoses, cell lines, DNA and some genotypes. A pilot study on Plains Indian subjects (N=69), by M-A. Enoch et al., detected LVA probands in five large pedigrees within what is essentially one very large tribal pedigree. Collection of this neurophysiological phenotype, relevant to alcoholism, will add to our ability to find alcoholism-vulnerability genes in these populations and to understand the gene/environment interaction in alcoholism vulnerability.

POPULATION GENETICS

The two focuses of population genetic research in LNG are: 1) the pattern of variation in populations of individual candidate gene polymorphisms whose origins and roles in behavior we need to better understand; and, 2) the genetic architecture of the Finnish and American Indian population isolates which are key to our genetic linkage studies.

$ALDH_2$: The discoveries that the $ALDH_2$ polymorphism, Glu487Lys, and the ADH_2 polymorphism, Arg47His, affect alcoholism vulnerability are salient achievements of the field which encourage attempts to identify additional vulnerability alleles. Existence of Lys487/Lys487 homozygotes with very low or nonexistent $ALDH_2$ enzyme activity raises questions as to the natural role of the functional enzyme, the forces that have brought the nonfunctional 487Lys allele to high frequency in East Asia and the consequences of Lys487 for alcohol preference and response. The evolutionary origin of Lys487, including the timing of its origin (coalescence time) in human populations, was defined in our lab (by R. Peterson et al.) by identifying multiple unique sequence variants at $ALDH_2$ followed by a cladistic analysis across multiple populations. The results are consistent with a single origin of Lys487 for the modern populations studied.

Genetic variation and population history - autosomal: Studies on autosomal genetic variation (by M.Urbanek, J. Long et al.) revealed that the American Indian populations we tested showed moderately reduced heterozygosity across a large panel of highly informative, short-tandem, repeat markers as compared to cosmopolitan Caucasian population. Although Finns, a well-defined population that is considered to be an isolate, did not show such a reduction in diversity. This study is the first to provide evidence that the population history of American Indians, including the dramatic 19th Century reductions in their numbers, has left a genome-wide imprint on diversity.

Genetic variation and population history - Y chromosome and mitochondrial: A study, by R.Kittles and J. Long et al., revealed that although autosomal diversity in Finns was unperturbed, the unique genetic history of Finland has left its imprint on Y chromosome diversity and haplotype pattern. In contrast to autosomal and mit-DNA variation, Y haplotype diversity was greatly reduced, indicating an effect specific to males (potentially the Thirty-Year War in which Finland was depopulated of males who fought in the Swedish army).

Integration of population and behavioral genetics - Y chromosome association to alcoholism in Finns: R.Kittles et al. used the knowledge we developed on Y chromosomal lineages in Finland to objectively group haplotypes for behavioral genetic association studies. Using methods originally developed by A. Templeton, Kittles et al. found that a specific Finnish Y chromosomal clade was associated with risk of alcoholism vulnerability. These particular Finnish Y chromosomes are, therefore, important targets for screening studies on the small number of genes which are found on the Y chromosome and which can potentially affect behavior.

Integration of population and behavioral genetics - $ALDH_2$: The discoveries that the $ALDH_2$ polymorphism, Glu487Lys, and the ADH_2 polymorphism, Arg47His, affect alcoholism vulnerability are salient achievements of the field which encourage attempts to identify additional vulnerability alleles. Existence of Lys487/Lys487 homozygotes, with very low or nonexistent $ALDH_2$ enzyme activity, raises questions as to the natural role of the functional enzyme, the forces that have brought the nonfunctional 487Lys allele to high frequency in East Asia and the consequences of Lys487 for alcohol preference and response. The evolutionary origin of Lys487, including the timing of its origin (coalescence time) in human populations, was being defined by R. Peterson et al. who identified multiple unique sequence variants at $ALDH_2$ followed by a cladistic analysis across multiple populations. The results are consistent with a single origin of Lys487 for the modern populations studied.

GENE EXPRESSION

$ALDH_2$: J. Jeng et al. have prepared transgenic mice, expressing either human $ALDH_2$ Glu487 or Lys487 mRNA, in order to better understand the role of $ALDH_2$ and its variants in ethanol preference, ethanol toxicity and metabolism. Multiple transgenic strains were prepared so that different levels of expression could be achieved by backcrossing animals in which the gene has inserted at different sites. Knockout mice for $ALDH_2$ are also being prepared. A strain, expressing the human $ALDH_{22}$ allele, shows the mRNA in multiple tissues, reduced enzyme activity and (in collaboration with P. Eriksson) higher tissue acetaldehyde levels after ethanol administration.

TPH: From heritability studies in humans and in rhesus macaque monkeys (Higley et al.), it is known that variation in serotonin function and, more precisely, CSF 5-HIAA is substantially heritable. However, defined environmental influences, e.g., isolation or change in dominance status, are capable of influencing serotonin function and consequent behaviors. Therefore, it is important to elucidate the molecular and cellular adaptive mechanisms by which function is altered. One key target is the promoter for the gene for TPH, since this is the rate-limiting enzyme in serotonin synthesis. Dr. Nielsen has elucidated the role of particular TPH promoter elements (four Sp1 sites and an RBP-Jκ site). Working with A. Rotondo, he also identified three polymorphism in the TPH promoter region which will be used for haplotype association and explored for potential effects on transcription.

DNA and protein damage and repair: Oxidative damage to DNA occurs due to alcohol intake. Dr. Brooks et al. established the presence of four DNA repair pathways in adult brain. It is possible that individuals with functional genetic variation in these pathways may be at greater vulnerability for alcohol-associated damage to nuclear DNA which, under ordinary circumstances, is largely repaired. This hypothesis is being explored in gene knockout mice. It is thought that a crucial step in the process of alcohol-associated protein and DNA-damage is the induction by alcohol of cytochrome P450 CYP2E1 leading to the generation of free radical metabolites and reactive oxygen species. Lipid-peroxides, such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA), are directly produced by CYP2E1 and are highly cytotoxic, probably because they directly react with proteins and DNA. For these reasons, Dr. Brooks has developed cell lines, deficient in nucleotide excision repair but rich in CYP2E1activity, by using a retroviral vector containing CYP2E1(from Dr. Arthur Cederbaum, Mount Sinai).

An understanding of control of expression of the alcohol-inducible CYP2E1 could lead to a better understanding of mechanisms of vulnerability to alcohol during fetal development and in adult tissues. Dr. Song et al. have shown that increased CYP2E1 expression is mainly due to enhanced enzyme stability via a blockade of the ubiquitin-mediated cytosolic degradation pathway. These results have resolved the controversy over the mechanism by which ethanol induces CYP2E1 and raises the issue as to whether other P450 enzymes are similarly regulated and the mode of ethanol's action on ubiquitin-mediated degradation.

Dr. Song's work also revealed that YH439, a synthetic thiazolium compound, appears to limit ethanol-associated hepatoxicity by inhibiting CYP2E1 transcription. The potential beneficial effects of YH439 are being evaluated in rodent prenatal and postnatal models of alcohol exposure. Using polyclonal antibodies against acetaldehyde, MDA and HNE protein adducts,

Song and colleagues quantitated the production of adducts in fetal tissues of control and alcohol-treated animals with and without pretreatment of YH439. Dr. Lee has also attempted to clone genes involved in the pathogenesis of fetal alcohol syndrome (FAS) using a differential display approach and identified one of these as fetal tropomyosin.

Toward understanding the role of the serotonin transporter in behavior, including alcohol intake, Drs. Ni, Brooks and colleagues have recently achieved the transient transfection of neurons in tissue culture and, in the brain, using a HSV vector containing this gene. Very high levels of functional expression of the serotonin transporter and transfection efficiency were reached. This technique offers a unique opportunity to increase serotonin transporter function in specific brain regions and at particular points in development . Their studies have now moved forward to the microinjection of specific brain regions to be followed by neurochemical and behavioral studies.

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INTRAMURAL RESEARCH PROJECT ZO1 AA 00019-05 LNG
October 1, 1996 to September 30, 1997

Title of Project: $ALDH_2$ Deficiency: Population Genetics and Relationship to Phenotype

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: M-A. Enoch, M.D., LNG
J. Long, Ph.D., LNG
R. Peterson, M.S., LNG

Collaborating Units: Laboratory of Parasitic Diseases, NIAID, NIH (T. Nutman, M.D.; P. Zimmerman, Ph.D.); Pulmonary-Critical Care Medicine Branch, NHLBI, NIH (A. Novoradovsky, Ph.D.)

Staff-Years: 1

Sample Type: Human tissues

Summary of Work: In Orientals, $ALDH_2$ deficiency, due to a common polymorphism, frequently causes a flushing reaction after alcohol consumption and this aversive reaction is responsible for lower rates of alcoholism in individuals with the inactive $ALDH_{22}$ allele. $ALDH_2$ deficiency was detected in South American Indian populations; however, these findings have never been confirmed and a previous search for the Oriental $ALDH_{22}$ allele in South American Indians was negative. Recently, R. Peterson has identified a series of additional markers at $ALDH_2$. By restriction enzyme analysis, single-strand conformational polymorphism (SSCP) and sequencing, he was able to identify haplotypes characteristic for the $ALDH_{22}$ and $ALDH_{21}$ alleles in different populations. This analysis is shedding light on the origins and functional role of the variant $ALDH_{22}$ allele, which appears to have surfaced on a single haplotype lineage and spread among East Asian populations. Although $ALDH_{22}$ R [Glu487Lys] probably originated on a single genetic background, haplotype analysis reveals that it is sufficiently ancient for additional mutations to have occurred subsequently. This result is most compatible with an effect of selection to maintain the Oriental $ALDH_2$ variant, Glu487Lys.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00280-08 LNG
October 1, 1996 to September 30, 1997

Title of Project: Genetics of EEG and ERP Traits Related to Alcoholism

Principal Investigator: M-A. Enoch, M.D. (Adjunct Investigator)
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Other Personnel: D. Goldman, M.D., LNG
C. Harris, LNG
R. Robin, Ph.D., LNG
K. White, M.Ed., LNG

Collaborating Units: Dept of Psychiatry,Washington University (J. Rohrbaugh, Ph.D.)

Staff-Years: 2.25

Sample Type: Human subjects (Interviews)

Summary of Work: Alcoholism is a common heterogenous disease. Heritability has been established in both men and women, but as for other psychiatric diseases, it has proved difficult to map genes directly for reasons such as genetic heterogeneity, phenocopies, penetrance and expressivity and polygenic effects. We have employed an indirect approach by identifying a trait-specific marker for alcoholism vulnerability, the low voltage α (LVA) EEG, a normal variant of the resting EEG in which the α rhythm is virtually absent. This phenotype is heritable, reproducible in each individual, present in 4-11% of the population and accurately determined. We now have a complete dataset, including EEG and ERP phenotypes, blind-rated DSM-III-R diagnoses, psychometric tests and DNA on 250 individuals. We have recently replicated the finding of our original study (Enoch et al., 1995) in a comparable sample of subjects and have found the same result in the group of 117 unrelated individuals from the total sample, namely that there is a phenotype-phenotype association between LVA EEG and a subtype of alcoholism that is associated with anxiety disorders. LVA was the EEG phenotype of 24% of the alcoholics, 36% of those with an anxiety disorder and 75% of the alcoholics with an anxiety disorder as compared to 8% of the individuals without alcoholism or an anxiety disorder. In order to have sufficient power to map genes for alcoholism, the focus of this study has shifted to a Plains American Indian tribe which has a high prevalence of alcoholism. Thus far, we have an extensive dataset (psychiatric diagnoses and DNA) on 350 tribal members from large pedigrees; an EEG pilot study conducted on 69 of these individuals showed a high prevalence of the LVA phenotype.

Formerly titled "Genetics studies of the electroencephalogram and event-related potentials."

INTRAMURAL RESEARCH PROJECT ZO1 AA 00281-08 LNG
October 1, 1996 to September 30, 1997

Title of Project: Molecular Genetic Studies on Alcoholism in American Indians – Southwestern Tribe

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: L. Akhtar, M.S., LNG
J. Long, Ph.D., LNG
R. Robin, Ph.D., LNG
M. Urbanek, Ph.D., LNG

Collaborating Units: NIDDK, NIH (W. Knowler, M.D.; P. Bennett, M.D.; R. Hanson, M.D.)

Staff-Years: 4

Sample Type: Human tissues

Summary of Work: To identify alcoholism vulnerability and protective genes, we collected and tested, for linkage and pattern of genetic transmission, families from American Indian populations. Such populations are relatively homogeneous genetically and environmentally. This report addresses an American Indian tribe in which alcoholism is highly prevalent. A total of 582 subjects from a large family genealogy were psychiatrically interviewed (SADs-L), blind rated for diagnosis and genotyped. An analysis (J. Long) of the familiality of alcoholism revealed a significant increase in relative risk in first-degree relatives of alcoholics. In females, the risk was highest in first-degree relatives and still significant at the 3rd degree of genetic relationship. Binge drinking was evaluated with the result that this pattern of behavior was not, as has been alleged, benign or beneficial, but associated with dramatic increases in problems in multiple domains: social, violence/lawlessness, work and medical. Transmission analysis in the Southwestern Indian families showed, for the first time, that alcoholism in an American Indian tribe is familial, despite the high rate of alcoholism in this tribe (more than 50% of females and more than 85% of males). A whole autosome genetic linkage analysis on a subset of the sample utilized 517 short tandem repeat markers. By sib-pair analysis, strong evidence for linkage to alcohol dependence (DSM-III-R) was found near the chromosome 11p telomere (near the DRD_4 dopamine receptor locus) and the centromeric region of chromosome 4p (near the GABA receptor cluster). Simulation analyses confirmed that these linkage signals were statistically highly significant and they could, on a conservative basis, have been randomly expected in about one in six and one in three whole genome linkage analyses, respectively. A more modest linkage signal was also detected on chromosome 4q (at the location of the alcohol dehydrogenase gene cluster).

INTRAMURAL RESEARCH PROJECT ZO1 AA 00290-07 LNG
October 1, 1996 to September 30, 1997

Title of Project: Molecular Genetic Studies of Disturbed Serotonin Function

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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J. Long, Ph.D., LNG
D.A. Nielsen, Ph.D., LNG
M. Okada, Ph.D., LNG
J. Lappalainen, M.D., Ph.D., LNG
C. Mazzanti, Ph.D., LNG
M. Linnoila, M.D., Ph.D., LCS
F.F. Weight, M.D., LMCN

Collaborating Units: University of Helsinki (M. Virkkunen, M.D.; M.Eggert, M.D.); Washington University (M. Pranzatelli); Institute of Behavior and Genetics (T. Johnson); University of Pittsburgh Medical Center (W. Kaye, M.D.); NCI, LVC (M. Dean, Ph.D.); NIMH (D. Murphy, M.D.; N. Rosenthal, M.D.)

Staff-Years: 3

Sample Type: Human tissues

Summary of Work: Studies on individuals and animals with genetic defects in serotonin function can shed light on the role of this neurotransmitter in behavior and on the role of milder functional variants in serotonin genes in predisposing individuals to psychopathologies and to alcoholism. We are identifying probands for family studies by measuring the serotonin metabolite 5-HIAA in cerebrospinal fluid and by identifying individuals with amino acid substitutions in genes involved with serotonin function. Two $5HT_{1A}$ variants are rare amino acid substitutions (Gly22Ser and Val28Ile), one conservative and one nonconservative. The $5HT_{2C}$ variant is a common (allele frequency=0.18) nonconservative substitution (Cys23Ser). Two $5HT_{2A}$ amino acid substitutions (Ala477Val and His452Tyr) have allele frequencies of 0.01 and 0.09. Rare serotonin transporter and $5HT_7$ amino acid substitutions were also discovered. Three of these amino acid substitutions were shown to alter the functional properties of the corresponding receptor. $5HT_{1A}$ Gly22Ser when expressed in CHO-K1 cells dramatically altered desensitization and down regulation of these receptors. $5HT_{2C}$ Cys23Ser in oocytes and COS-7 cells decreased ligand binding and $5HT_{2A}$ His452Tyr impaired signal transduction in platelets from subjects with the 452Tyr allele.

For association and direct gene analysis, we have collected more than 40 cell lines from each of the following populations: anorexia nervosa (collaboratively with W. Kaye), obsessive compulsive disorder (D. Murphy), low CSF 5-HIAA with type II alcoholism (M. Linnoila, M. Virkkunen, M. Eggert), and seasonal affective disorder (N. Rosenthal, N. Ozaki). The detected polymorphisms are converted to PCR RFLPs or allele-specific amplification markers for ease of analysis. Using the CEPH reference pedigrees and the polymorphisms at these genes, each gene is genetically mapped to its chromosomal location. For direct gene analysis, we mainly use single-strand conformational polymorphism analysis and direct sequencing.

Association of a TPH polymorphism with suicidality in impulsive alcoholic Finns was replicated. Sib-pair linkage of $5HT_{1B}$ to antisocial alcoholism was found in Finns (J. Lappalainen) and replicated in Southwestern American Indians. Finally, the serotonin transporter promoter variant, 5HTTLPR, which was previously linked to neuroticism was linked to the two anxiety-related subscales of the TPQ in a sib-pair analysis (C. Mazzanti), partially replicating an earlier finding.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00291-03 LNG
October 1, 1996 to September 30, 1997

Title of Project: *In Situ* Reconstitution of Variants of Human 5HT Receptor Subtypes

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: M. Okada, Ph.D., LNG
M. Linnoila, M.D., Ph.D., LCS

Collaborating Units: Laboratory of Cell Biology, NIMH, NIH (J. Northup, Ph.D.)

Staff-Years: 3.0

Sample Type: Human tissues

Summary of Work: 5HT is a neurotransmitter that mediates a diverse array of physiological responses by interacting with multiple 5HT receptor subtypes. At least 14 5HT receptor genes have been cloned and we have identified variants in genes of several 5HT receptor subtypes ($5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_{5A}$, $5HT_6$ and $5HT_7$). Relationship between the genotypes, behavior and biochemistry is under investigation. However, the effect of variation in a single allele is obscure because of neuroadaptive processes and interaction with other genetic and environmental factors. Existence of variants resulting from alternative splicing or RNA editing might also complicate evaluation of the involvement of genotypes of 5HT receptors in behavioral phenotypes. Therefore, it is important to demonstrate the functional variants in genes that control serotonergic signal transduction.

Recently, Dr. Northup has established a method to reconstitute rat $5HT_{2C}$ 1G protein complexes *in vitro*. This system enables us to evaluate the functional properties of two common $5HT_{2C}$ receptor alleles (Cys23 and Ser23) including their interaction with G protein subunits (paper in preparation). Additional baculoviral vectors have been prepared to explore, in this reconstituted system, the functional properties of three variants of $5HT_{1A}$ and three variants of $5HT_{2A}$ receptors to reconstitute these receptors.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00293-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Role of the Serotonin Transporter Promoter Polymorphism in Psychiatric Disorders

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: J. Lappalainen, M.D., Ph.D, LNG
J. Long, Ph.D., LNG
C. Mazzanti, Ph.D., LNG
A. Rotondo, M.D., LNG
M. Linnoila, M.D., Ph.D., LCS

Collaborating Units: University of California (C. Reist, M.D.); University of Pisa (G. Cassano, M.D.); University of Helsinki (M. Virkunnen, M.D.); NIMH (D. Murphy, M.D.; N. Rosenthal, M.D.; A. Malhotra, M.D.; D. Pickar, M.D.; A. Heinz, M.D.)

Staff-Years: 1.0

Sample Type: Human subjects (Interviews)

Summary of Work: Dysfunctions in serotonergic pathways may underlie several psychiatric disorders. The serotonin transporter (5HTT) plays a critical role in the termination of serotonergic neurotransmission by Na-dependent uptake of serotonin by the presynaptic neuron. 5HTT also represents the initial site of action of certain antidepressant drugs and neurotoxins. A functionally significant polymorphism in the 5HTT promoter was identified (5HTTLPR). The polymorphism affects 5HTT transcription and, ultimately, 5HTT function.

Frequency of the 5HTTLPR was determined in a variety of clinical psychiatric populations including alcoholics; linkage and association studies were performed. Positive linkage was detected between 5HTTLPR and the two anxiety-related personality traits available on the Tridimensional Personality Questionnaire (TPQ), at least, partially replicating the reported association of this variant to behavior (see bibliography). In contrast, no association was found in Italian patients with obsessive compulsive disorders, panic disorders and eating disorders. However, two additional disease-specific findings were made: 1) in a collaboration with A. Malhotra and D. Pickar, 5HTTLPR was found to be significantly associated with BPRS-rated psychoticism in schizophrenia; and, 2) in a collaboration with N. Rosenthal, HTTLPR was significantly linked with seasonal affective disorder and seasonality rating in SAD patients.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00294–01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Evolution and Variation of Macaca $5HT_{1A}$

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: A. Bergen, Ph.D., LNG
J.D. Higley, Ph.D., LCS
D.A. Nielsen, Ph.D., LNG
M. Pratt, LNG

Collaborating Units: LABS, Inc. (P. Mehlman, Ph.D.); UCLA (M. Raleigh, Ph.D.)

Staff-Years: 1.0

Sample Type: Neither human subjects nor tissues

Summary of Work: $5HT_{1A}$ is the intronless coding locus (1266 base pair - 422 amino acids) for a G protein-coupled serotonin receptor with a typical 7-transmembrane structure, located on chromosome 5 in humans. Previous work in this laboratory discovered two variants (Biochem Biophys Res Commun 1995;210(2):530-6), characterized their frequency and distribution in human populations (Human Mutation 1996;7:135-43) and investigated their functional effects (Neuropsychopharmacology 1997;17:18-26). In order to assess the polymorphic spectrum of this locus in a primate animal model heavily used in neuroscience research, we have cloned and sequenced the highly conserved $5HT_{1A}$ gene from four macaque species (Macaca fascicularis , Macaca maura, Macaca mulatta and Macaca nemestrina) and from the vervet monkey (Cercopithecus aethiops). Both interspecific and intraspecific sequence variations have been discovered, the interspecific variation supporting the known phylogeny of Macaca, while the intraspecific variation will be characterized in a large group of Macaca mulatta for which serotonin metabolites and behavioral data exist in order to assess potential association between serotonin receptor variants and behavior.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00295-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Y Chromosome Population Genetics

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: R. Aragon, LNG
A. Bergen, Ph.D., LNG
J. Long, Ph.D., LNG
R. Kittles, M.S., LNG
J. Kokoszka, LNG

Collaborating Units: Yale University (K. Kidd, Ph.D., J. Kidd, Ph.D.); Natal Institute of Immunology (M. Hammond, Ph.D.); University of California, Berkeley (W. Klitz, Ph.D.); NINDS, NIH (L. Goldfarb, M.D.)

Staff-Years: 1.5

Sample Type: Human subjects

Summary of Work: The estimation of the mutation rates of Y chromosome microsatellite loci, human Y chromosome phylogeny and human population divergence dates are the goals of this project. Approximately 500 individuals, drawn primarily from 5 Asian and 11 Native American population samples, are being genotyped at 9 microsatellite loci and 5 non-repetitive loci with known ancestral state, all located on the non-recombining, non-pseudoautosomal region of the human Y chromosome. Haplotypes will be constructed from the collected genotypes and population genetic parameters, including heterozygosity and allelic repeat unit variance statistics, will be calculated to compare population diversities and haplotype/population associations. Phylogenetic analysis using distance (population variance) and parsimony (interhaplotypic distance) methods will construct networks of evolutionarily-related populations and haplotypes. Linkage disequilibrium statistics will be calculated to estimate microsatellite mutation rates using population modeling approaches adapted from autosomal linkage disequilibrium mapping methods. These analyses will contribute to a understanding of the dispersal and migration of ancestral Asian populations into Asia and the Americas and will describe the relationships among descendent populations through the combined population genetic, phylogenetic and linkage disequilibrium analyses performed.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00296-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Alcohol Dependence and Chromosome 11p15.5

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Bethesda MD 20892

Other Personnel: A. Bergen, Ph.D., LNG
J. Long, Ph.D., LNG
D. McKeane, LNG
R. Robin, Ph.D., LNG
L. Wilhelm, LNG

Collaborating Units: NIMH, NIH (S. Kim, M.S.)

Staff-Years 1.0

Sample Type: Human subjects (Interviews)

Summary of Work: In order to follow-up a linkage finding on chromosome 11p15.5 to alcohol dependence (Long et al., submitted), two short tandem-repeat marker panels for semi-automated fluorescent genotyping containing 15 loci distributed over the distal 20 cM of chromosome 11p15.5 have been created. One panel (six loci) has been typed in approximately 500 psychiatrically-interviewed individuals from a Southwest American Indian tribe. A second panel (nine loci) has been optimized and typing will begin soon. In addition, two coding polymorphisms and one promoter polymorphism at DRD4, a candidate gene for involvement in vulnerability to alcohol dependence, have been typed in order to develop haplotypes at this locus, distal to the short-tandem repeat locus linked in the whole genome linkage scan. Haplotype construction, sib-pair linkage analysis and map construction will be performed to confirm the primary linkage finding and define intervals of maximum linkage to alcohol dependence.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00297-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Evolution and Variation of RPS4Y

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Bethesda MD 20892

Other Personnel: A. Bergen, Ph.D., LNG
K. Jefferson, LNG
C. Wang, LNG

Collaborating Units: Johns Hopkins University (K. Smith, Ph.D.); Seoul National University (S. Park, Ph.D.); Taipei Blood Center (S. Tsai, Ph.D.); LABS, Inc. (P. Mehlman, Ph.D.); NCI, NIH (S. O'Brien, Ph.D.); NINDS, NIH (L. Goldfarb, M.D.)

Staff-Years: 2.0

Sample Type: Human subjects

Summary of Work: Sequence variation within the non-pseudoautosomal region of the Y chromosome within and between the Hominidae can elucidate Y chromosome evolution and paternal human population history. The sequence of the coding region of the RPS4Y locus, a ribosomal protein gene, was determined in 4 non-human primate species and in 59 individuals from three human populations. Sequence analysis of RPS4Y in the Hominidae suggests that the RPS4Y protein is under relaxed functional selection compared to its highly conserved homolog, RPS4X, and predicts that the gene transposed to the Y chromosome approximately at the prosimian-simian divergence. Sequence variation at RPS4Y was detected both within and between human populations. One RPS4Y variant, C711T, appears to be the first common coding sequence polymorphism on the Y chromosome. The coalescent of human sequences (175,000 ± 125,000 years) at this locus is similar to estimates derived from different Y loci and different human population samples. The ethnographic distribution of this Y chromosome substitution identifies a paternal lineage ancestral to Asian and Native American populations.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00298-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Mu Opioid Receptor Polymorphisms and Alcohol Dependence

Principal Investigator: D. Goldman, M.D. (Senior Investigator)
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Other Personnel: A. Bergen, Ph.D., LNG
J. Kokoszka, LNG
M. Linnoila, M.D., Ph.D., LCS
J. Long, Ph.D., LNG
R. Peterson, M.S., LNG

Collaborating Units: University of Helsinki (M. Virkkunnen, M.D.)

Staff-Years: 1.5

Sample Type: Human subjects (Interviews)

Summary of work: The μ opioid receptor is implicated in the reward, tolerance and withdrawal effects of alcohol and other drugs of abuse. We directly sequenced the human μ opioid receptor locus, OPRM1, to detect natural variation that might affect the function of this receptor or be associated with psychiatric phenotypes related to opioid function. Four DNA sequence variants were found: three amino acid substitutions (Ala6Val, rare; Asn40Asp, frequency 10%; Ser147Cys, rare) and one intronic variant (IVS2+691G/C, frequency 50%). OPRM1 alleles, genotypes and haplotypes from three psychiatrically characterized population samples (N = 791) were used to perform association and sib-pair linkage analyses to alcohol dependence. There was no significant association or linkage between OPRM1 and alcohol dependence in any of the three population samples. These results and power calculations strongly suggest that variation at the μ opioid receptor is not involved in vulnerability to DSM-III-R alcohol dependence. Variation at this gene could be investigated for possible association to response to opiate pharmacotherapy and to variation in opioid function, that is, pain or nociception and the regulation of hypothalamic-pituitary function.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00299-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Tryptophan 2,3-Dioxygenase; a Candidate Gene for Disorders of Serotonin Metabolism

Principal Investigator: M-A. Enoch, M.D. (Adjunct Investigator)
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Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG
M. Linnoila, M.D., Ph.D., LCS

Collaborating Units: University of Pittsburgh Medical Center (W. Kaye, M.D.); University of Chicago (E. Cook, Ph.D.); McGill University (R. Palmour, Ph.D.); University of Pisa (G. Cassano, M.D.); University of Helsinki (M. Virkkunen, M.D.); NIMH, NIH (D. Murphy, M.D.)

Staff-Years: 0.75

Sample Type: Human tissues

Summary of Work: Genetic defects in the enzymes involved in serotonin metabolism may be implicated in the causation of a wide range of diseases, from eating disorders, obsessional compulsive disorder and alcoholism to autism. Tryptophan, obtained only from the diet in humans, is converted to serotonin by tryptophan hydroxylase, or to kynurenine by tryptophan 2,3-dioxygenase (TDO_2). Both enzymes are rate limiting in their respective pathways.

The purpose of this study is to screen the TDO_2 gene for polymorphisms, assess functionality, and search for disease associations in 350 individuals, primarily using single-strand conformational polymorphism (SSCP) analysis. Most of the coding region (11 of the 12 exons) and short regions of the introns was successfully amplified and screened across populations with anorexia or bulimia nervosa, obsessive-compulsive disorder, autism, major depression and suicidality, impulsivity and alcoholism, and subjects enrolled in a tryptophan depletion study. No associations were found for polymorphisms in introns 5 and 6, nor for a mutation in exon 7 (A to C, 749 Asn to His). The promoter region is being screened, no polymorphisms were found in the two TATA boxes, and in the putative glucocorticoid site an A to C variant was detected but with no disease association. However, in the promoter region of GTT repeats, an SSCP variant has been found which, on polyacrylamide gel electrophoresis, manifests both a long and short allele, implying an insertion. This region is currently being sequenced. This variant appears to be associated with impulsivity. A sib-pair analysis of 600 individuals is underway to confirm this finding.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00036-11 LNG
October 1, 1996 to September 30, 1997

Title of Project: Regulation of Biological Roles of Ethanol-Inducible Cytochrome P450 2E1 (CYP2E1)

Principal Investigator: B-J. Song, Ph.D. (Senior Investigator)
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Other Personnel: A. Kallarakal, Ph.D., LNG
J. Lee, Ph.D., LNG
Y. Soh, Ph.D., LNG

Collaborating Units: Genetic Research Institute, Korea (K. Jeong, Ph.D.)

Staff-Years: 3.0

Sample Type: Neither human subjects nor tissues

Summary of Work: We have demonstrated multiple regulatory mechanisms for CYP2E1: induction via transcription, mRNA stabilization, activation of mRNA translation and protein stabilization, suppression via transcription, mRNA and protein degradation. We recently reported transcriptional suppression of CYP2E1 gene by an exogenous compound, YH439, and the potential beneficial effects of this synthetic inhibitor were studied in an *in vivo* model of acute hepatitis by treatment with carbon tetrachloride. *In vivo* hepatobiliary imaging analyses revealed that YH439 efficiently protects liver injury from carbon tetrachloride. These results were confirmed by the corresponding changes in the levels of serum transaminases and by histological evaluations. The protective effect of YH439 appears to be the result of effective suppression of CYP2E1, which catalyzes the metabolism of carbon tetrachloride, leading to the initiation of free-radical mediated tissue damage. The level of acetaldehyde adduct was also studied. Our immuno-blot data, using polyclonal antibody against acetaldehyde-protein adduct, showed that an immunoreactive band (apparent Mr37kDa on SDS-polyacrylamide gel) was not detected in animals treated with control diet while it was clearly detected in rats pair-fed with alcohol liquid diet. However, it was virtually absent upon treatment with YH439. Immunocytological analyses revealed that immunoreactive antigen is primarily found in the pericentral region where CYP2E1 is mainly localized. These data suggest that the acetaldehyde-protein adduct (Mr37kDa) was produced in a CYP2E1-dependent manner. Since CYP2E1-mediated metabolism is known to cause oxidative stress, resulting in DNA and protein damage, the levels of DNA adducts in rat tissues from different treatments, including ethanol in the absence and presence of YH439, are being measured by HPLC. We have also searched for potential mutations in the human CYP2E1 gene, by analyzing DNA samples obtained from individuals with low and high levels of CYP2E1 activity. Our data suggest that different levels of CYP2E1 activity do not correspond with CYP2E1 gene polymorphism. In addition, over-production of CYP2E1 in baculovirus expression system is being performed to study the biological role of each of the four exposed lysine residues, as viewed in a model CYP2E1 protein structure. These lysine residues are good candidates for ubiquitin conjugation, leading to the proteosomal degradation of CYP2E1; each is being mutated with alanine to study rates of ubiquitin conjugation and subsequent CYP2E1 degradation and to elucidate the exact mechanism of CYP2E1 stabilization by ethanol.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00090-03 LNG
October 1, 1996 to September 30, 1997

Title of Project: Functional Role of $ALDH_2$ Using Transgenic Mice Carrying the Asian Variant Allele ($ALDH_{22}$)

Principal Investigator: B-J. Song, Ph.D. (Senior Investigator)
LNG, DICBR, NIAAA, NIH
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Other Personnel: D. Goldman, M.D., LNG
J. Jeng, Ph.D., LNG

Collaborating Units: Laboratory of Molecular Carcinogenesis, NCI, NIH (F. Gonzalez, Ph.D.); Laboratory of Nutritional and Molecular Regulation, NCI, NIH (T. Wang, Ph.D.)

Staff-Years: 0.5

Sample Type: Neither human subjects nor tissues

Summary of Work: The mitochondrial, aldehyde dehydrogenase ($ALDH_2$), is the major ALDH isozyme involved in acetaldehyde metabolism. It is well established that a single nucleotide substitution (G to A) which results in the amino acid change (Glu487Lys) leads to dominant inactivation of $ALDH_2$ activity. This genetic polymorphism is the cause of the flushing response observed in many Asian people following alcohol intake. Although the $ALDH_{22}$ allele has been shown to have a protective role against alcoholism, the physiological role of this enzyme is still unclear. To further examine the possible physiological role of $ALDH_2$, we produced transgenic mice carrying the human $ALDH_2$ variant ($Haldh_{22}$). Currently, we have established two independent lines of $Haldh_2$ transgenic mice. These mice were used to study the effects of $Haldh_2$ on alcohol preference, metabolism of endogenous and exogenous substrates, behavior and tissue damage after long-term alcohol consumption. Human $ALDH_2$ protein was expressed in all tissues examined and expression of human $ALDH_{22}$ inhibited mouse $ALDH_2$ enzyme activity in transgenic mice. Mice were injected with 20% ethanol ip for over two weeks. We observed that fubin background strain mice showed fear, avoidance and escape behavior after ethanol-treatment but these changes in behavior were not evident in the transgenic mice exposed to ethanol ($p<0.001$). To correlate the apparent behavioral change with levels of neurotransmitters and to study the role of $ALDH_2$ in endobiotic metabolism, the levels of various monoamine neurotransmitters were determined by HPLC. In brain, dopamine and its metabolite, 3.4-dihydroxyphenyl acetic acid (DOPAC), were significantly elevated in FVB/N mice treated with ethanol. In contrast, this elevation was absent in transgenic mice. Brain serotonin level was also elevated by ethanol treatment but to a lesser extent than dopamine level. Brain norepinephrine was unchanged in any of the strains. These results suggest that behavioral differences in the transgenics are due to alteration in monoamine metabolism by the introduction of $Haldh_{22}$. Our preliminary data also indicate that female transgenic mice consume 40% less alcohol ($p<0.0001$) than do FVB/N background mice in a two-bottle choice paradigm. No significant difference was observed in male transgenic mice. These data indicate that transgenic mice carrying the $Haldh_{22}$ can be a valuable model to study the role of ALDH in behavior, neurotransmitter metabolism and drinking preference.

This project was formerly titled "Physiological Role of the Oriental Variant of $ALDH_2$ ($ALDH_{22}$): Preparation of Transgenic and Knockout Mice"

INTRAMURAL RESEARCH PROJECT ZO1 AA 00100-02 LNG
October 1, 1996 to September 30, 1997

Title of Project: Computer Assisted Molecular Modeling

Principal Investigator: B-J. Song, Ph.D. (Senior Investigator)
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Other Personnel: D. Goldman, M.D., LNG
A. Kallarakal, Ph.D., LNG

Collaborating Units: Center for Molecular Modeling, DCRT (R. Pearlstein, Ph.D.; Z. Lin, Ph.D.)

Staff-Years: 0.5

Sample Type: Neither human subjects nor tissues

Summary of Work: Lack of structural information is a major stumbling block in the characterization of the majority of proteins and their naturally occurring variants. Modeling programs like Quanta, Insight and Look are useful in overcoming the above-mentioned problems, to a great extent, by providing tools to generate three-dimensional structures. We have initiated study on the structures of various membrane receptors and enzymes that are being investigated for genetic functional variation in the Laboratory of Neurogenetics. Structures of the transmembrane helical domains of various serotonin receptors are being generated using the electron density map of bovine rhodopsin as the template structure. Extra-cellular and intra-cellular loops having 16 or less amino acids will be added to this helical structure to arrive at a model for each of the serotonin receptors. Analyses of molecular dynamics, using the programs Charmm and Dock, will be carried out on these structures to understand ligand specificity and binding. In addition, homology modeling based on the crystal structure of yeast transketolase has been performed for the thiamin dependent enzymes, mammalian transketolase and the E1α and E1β subunit of pyruvate dehydrogenase complex, whose activities are reportedly reduced in several neurodegenerative diseases. The structures of the various naturally occurring variants observed in these enzymes are being compared to those of the native counterparts to delineate the roles of certain variant amino acids in the binding of substrates and cofactors as well as enzyme catalysis. Furthermore, computer-aided modeling can be utilized in the characterization of other proteins that are being investigated by the NIAAA.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00102-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Molecular Characterization of Fetal Alcohol Syndrome Pathogenesis

Principal Investigator: B-J. Song, Ph.D. (Senior Investigator)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: I. Lee, Ph.D., LNG
Y. Soh, Ph.D., LNG

Collaborating Units: None

Staff-Years: 2

Human subjects: Neither human subjects nor tissues

Summary of Work: Fetal alcohol syndrome (FAS) is one of the leading causes of mental retardation in the world. We have undertaken a molecular and biochemical approach towards understanding the pathogenesis of FAS. The technique of differential mRNA display was used to detect changes in gene expression in developing mouse embryos caused by ethanol exposure. Mouse embryos were also treated with another known teratogen, 3-methylcholanthrene (3-MC), in order to identify mRNAs which are specifically regulated by ethanol. Northern blot analyses were performed to confirm the differential display findings. We have identified two known and one unknown cDNA whose corresponding mRNA levels are altered by exposure to ethanol. A brain specific isoform of α-tropomyosin is up-regulated by ethanol but not by 3-MC in 11-day old embryos. Immunoblot analyses indicate that the level of the α-tropomyosin protein is also elevated by ethanol exposure. The brain specific isoform of α-tropomyosin has been shown to be important for central nervous system development and its ectopic expression during a critical period of development may disrupt normal development and cause some of the phenotypes seen in FAS. The other known cDNA encodes heat shock protein 47 (HSP47) which is involved in pro-collagen processing. The expression of the HSP47 gene was shown to be induced by ischemia in the adult rat brain. Ischemia has been noted as a possible mechanism of FAS as it has been shown that injection of bolus doses of ethanol into monkeys causes the collapse of the umbilical cord and decreased blood flow. We have recently used cDNA microarrays to identify additional mRNAs, which are altered during the early stages of FAS. The identification of these mRNAs will increase our understanding of the teratogenic actions of ethanol and the chances for effective treatment.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00008-05 LNG
October 1, 1996 to September 30, 1997

Title of Project: Studies on Serotonergic Gene Function and Behavior in Transgenic Mice

Principal Investigator: D.A. Nielsen, Ph.D. (Research Fellow)
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Other Personnel: D. Goldman, M.D., LNG
F.S. Hall, Ph.D., LCS
G. Jenkins, M.S., LNG

Collaborating Units: None

Staff-Years: 0.20

Human subjects: Neither human subjects nor tissues

Summary of Work: Since alcoholism is associated with decreased serotonin turnover, we have focused on genetic determinants of serotonergic behaviors to identify factors contributing to a predisposition to alcoholism. In neurons, of the raphe nuclei, serotonin biosynthesis is governed by the activity of the tryptophan hydroxylase (TPH) enzyme, which is rate-limiting. The cDNA and gene coding for murine TPH were previously cloned. These sequences have been combined with sequences from HSV thymidine Kinase and mouse metallothionein genes. These are being introduced into mouse embryos to create transgenic mice to study gene sequences controlling tissue-specific and developmental expression and to characterize effects of high and low TPH gene activity and ablation of TPH-expressing cells on behavior.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00086-04 LNG
October 1, 1996 to September 30, 1997

Title of Project: Molecular Studies on Genetic Variants of Serotonergic Genes

Principal Investigator: D.A. Nielsen, Ph.D. (Research Fellow)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: A. Rotondo, Ph.D., LNG
D. Goldman, M.D., LNG
G. Jenkins, M.S., LNG
L. Akhtar, M.S., LNG

Collaborating Units: UCLA (M. Raleigh, Ph.D.)

Staff-Years: 2.08

Sample Type: Human tissues

Summary of Work: CSF 5-HIAA, the principal metabolite of serotonin, can identify individuals with a deficit of central nervous system serotonin metabolism. Decreased serotonin metabolism, as indicated by low CSF 5-HIAA, is postulated to be connected with behaviors including alcoholics characterized by deficient impulse control. Trait differences in CSF 5-HIAA concentration may be due to genetic variants of genes regulating serotonin metabolism. To identify factors controlling serotonin-dependent behaviors, we have focused our efforts on tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of serotonin, and the 5HT receptor which, in part, regulates serotonergic activity.

The human TPH gene was mapped to chromosome 11p15.5, a human TPH variant in intron 7, associated with CSF 5-HIAA concentration in alcoholic, impulsive, Finnish offenders. The variant also associated with a history of suicidal attempts and of multiple suicidal attempts in alcoholic Finnish offenders. This finding of an association of the TPH variant with suicidal behavior has been replicated in a new population of Finnish alcoholic offenders. When we combine the two studies, the association of TPH to suicide history is significant in both the impulsive group and the total alcoholic offender sample. Linkage of TPH has been found to alcoholism, history of suicide attempts and of medically-damaging suicide attempts and to KSP socialization score. The TPH variants have been sequenced and no alteration in mRNA splicing was observed. Three additional TPH variants have been identified and are being characterized.

Three variants of the human $5HT_{1A}$ gene have been identified by single-strand conformational polymorphism (SSCP) analysis. Two variants change the protein sequence and the third is silent. The Ser22 variant in the extracellular amino-terminus of the human $5HT_{1A}$ receptor stably expressed in CHO-K1 cells decreases agonist-promoted down-regulation and desensitization of receptor expression. In addition, an allelic variant has been identified in the $5HT_{1A}$ gene in vervet monkeys. We are studying the relationship of the vervet variant to CSF 5-HIAA and prosocial behaviors.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00087-04 LNG
October 1, 1996 to September 30, 1997

Title of Project: Studies on DNA Single-Strand Conformation Prediction

Principal Investigator: D.A. Nielsen, Ph.D. (Research Fellow)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG
A. Kallarakal, Ph.D., LNG

Collaborating Units: Mayo Clinic (S. Sommer, Ph.D.); University Hospital of Obstetrics and Gynecology (A. Jordanova, Ph.D.)

Staff-Years: 0.18

Human subjects: Neither human subjects nor tissues

Summary of Work: To identify genetic contributions to alcoholism vulnerability, we have been using single-strand conformational polymorphism (SSCP) analysis. SSCP analysis identifies polymorphic alleles through the detection of altered single-strand DNA secondary structure. The altered secondary structure occurs due to nucleotide changes in DNA sequence. To aid in our understanding of the SSCP technique, we have devised a computer program, DNA-Fold Version 1, to emulate the folding of single-strand DNAs.

The efficiency of the DNA-fold program is being evaluated for its predication of SSCP mobility of several hundred DNA molecules. In an effort to improve the efficiency of the DNA-fold program, the DNA thermodynamics for the dangle stacking energies are being determined. These dangle energies and improved thermodynamic measurements that have recently become available are being evaluated in the DNA-Fold program to see if the efficiency of the program will be improved. Parameters will be modified to increase predictive efficiency under various conditions.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00234-15 LNG
October 1, 1996 to September 30, 1997

Title of Project: Molecular Studies on Serotonergic Gene Expression

Principal Investigator: D.A. Nielsen, Ph.D. (Research Fellow)
LNG, DICBR, NIAAA, NIH
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Other Personnel: D. Goldman, M.D., LNG
F.S. Hall, Ph.D., LCS
G. Jenkins, M.S., LNG
K. Schuebel, Ph.D., LNG

Collaborating Units: UCLA (M. Raleigh, Ph.D.); NCI, NIH (M. Dean, Ph.D.)

Staff-Years: 1.88

Sample Type: Human subjects

Summary of Work: To identify genetic contributions to alcoholism vulnerability, we focused on serotonergic behaviors, since a subtype of alcoholism is associated with decreased serotonin turnover. Serotonin biosynthesis is governed by tryptophan hydroxylase (TPH), which is rate-limiting. We hypothesize that factors controlling its gene expression play a major role in behavior.

The promoter elements controlling TPH gene expression have been analyzed through the use of gene fusions and EMSAs. Two regions necessary for high level transcription were identified; two sites in the upstream TPH promoter and a site in the 5' untranslated region bind Sp1. An upstream site was found to be required for repression in a cell-specific fashion and bound RBP-Jκ. TPH is the first mouse gene shown to be regulated by RBP-Jκ. RBP-Jκ probably repressed TPH transcription by quenching Sp1 activation through blockage of Sp1 function. A variant RBP-Jκ allele, carrying an amino acid substitution, located within the DNA-binding domain was discovered. High affinity binding sites for RBP-Jκ were identified among 5 introns (introns 1, 2, 6, 7 and 10) of the mouse TPH gene. Several RBP-Jκ were also found in the human TPH gene.

TPH gene expression has been studied *in vitro* to define the mechanisms controlling its regulation. We hypothesized a negative feedback-loop regulating serotonin production. TPH-luciferase constructs have been used to identify several agents which regulate TPH transcription. Furthermore, TPH mRNA was demonstrated in Neuro-2a cells, the first report in a neuronal line.

Experiments are in progress in the rat with direct injections of TPH and 5HT antisense oligothiophosphonucleotides directly into the raphe nucleus. The role of TPH and 5HT in alcohol consumption is being investigated.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00083-04 LNG
October 1, 1996 to September 30, 1997

Title of Project: DNA Damage, DNA Repair, and Alcohol

Principal Investigator: P.J. Brooks, Ph.D. (Research Fellow)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG
C. Marietta, M.S., LNG

Collaborating Units: Mt. Sinai School Med (A. Cedarbaum, Ph.D.); Rium, Leiden, The Netherlands (H. Van Steeg, Ph.D.); NIEHS, NIH (K. Tindall, Ph.D.; R. Sobol, Ph.D.); NCI, NIH (J. Robbins, M.D.)

Staff-Years: 3.0

Sample Type: Neither human subjects nor tissues

Summary of Work: Alcohol consumption produces a variety of pathological effects, including fetal alcohol syndrome, liver and brain damage and an increased risk of certain types of cancers. The association between ethanol consumption and cancer indicates that alcohol intake results in effects on genomic DNA. Mechanisms by which ethanol can produce DNA damage are: 1) direct adduction of DNA by acetaldehyde, the major metabolite of ethanol; 2) the generation of DNA damaging oxygen radicals via cytochrome P450 2E1 (CYP2E1), which is induced by ethanol in liver and brain. Our hypothesis is that genotoxicity occurs when the level of ethanol-induced DNA damage overwhelms the capacity of the relevant DNA repair systems. Thus the level of DNA repair activity in cells is a crucial determinant of alcohol-induced DNA toxicity.

Ongoing work focuses on a detailed understanding of DNA repair mechanisms in target tissues for ethanol toxicity, in particular, the brain. We have developed the first *in vitro* assay for the nucleotide excision repair (NER) pathway in adult brain tissue. NER repairs some types of oxygen radical damage to DNA and is critical for protecting neurons against endogenous oxidative DNA damage. Induction of CYP2E1 by ethanol would also result in increased levels of reactive oxygen species and oxidative DNA damage. This system is being used to better characterize the NER pathway in brain cells.

To better understand the role of different DNA repair pathways in addressing ethanol-induced DNA damage, we are examining the effects of ethanol, acetaldehyde and elevated levels of CYP2E1 on cellular toxicity in cell lines and whole animals lacking specific DNA repair pathways. These experiments will determine the relative role of the different DNA repair pathways in protecting cells against different types of ethanol-induced DNA damage. This work may have important implications for human beings with deficiencies in these pathways.

Another major focus is N2-ethyl deoxyguanosine, the major DNA adduct produced by acetaldehyde, which is undetectable in normal liver but accumulates in the DNA of alcohol-fed mice. We are assessing whether the adduct is a substrate for DNA repair and type of repair involved and its mutagenicity in mammalian cells.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00091-03 LNG
October 1, 1996 to September 30, 1997

Title of Project:	Genetic and Neurobiological Factors in an Animal Model of Antidepression
Principal Investigator:	P.J. Brooks, Ph.D. (Research Fellow) LNG, DICBR, NIAAA, NIH Bethesda MD 20892
Other Personnel:	D. Goldman, M.D., LNG
Collaborating Units:	None
Staff-Years:	0
Human subjects:	Neither human subjects nor tissues
Summary of Work:	This project has been terminated.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00101-02 LNG
October 1, 1996 to September 30, 1997

Title of Project: Manipulation of Specific Proteins Involved in Alcohol Intake and Behavior Using Herpes Viral Vectors

Principal Investigator: P.J. Brooks, Ph.D. (Research Fellow)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG
C. Marietta, M.S., LNG
Y. Ni, M.D., Ph.D., LNG

Collaborating Units: NIMH, NIH (B. Hoffman, M.D.)

Staff-Years: 0.4

Human subjects: Neither human subjects nor tissues

Summary of Work: There is abundant evidence linking brain monoamine neurotransmitter systems to behaviors such as alcoholism, aggression, reward, and psychiatric states including depression and schizophrenia. The evidence consists of correlations between monoamine levels and psychiatric disease and behavior in humans and drug effects in humans and experimental animals. The next step is to link specific molecules with behavioral effects. One approach to this issue is to use viral vectors to deliver constructs that will modulate the level of specific behaviorally relevant proteins *in vivo*. The approach is now used routinely to deliver therapeutic molecules to the brain. We are using neurotropic viral vectors to manipulate levels of endogenous molecules involved in monoamine neurotransmission (e.g., tyrosine hydroxylase, tryptophan hydroxylase, monoamine transporters). This is done by antisense constructs to reduce levels of specific molecules or by using constructs which will overexpress specific molecules. This approach will allow us to produce brain region-specific, reversible changes (either increases or decreases) in the level of specific molecules of interest and study the effects of such manipulations on alcohol intake and behavior.

We have constructed amplicon vectors which express sense or antisense RNA to tyrosine hydroxylase and the serotonin transporter under the control of the herpes virus a 4 promoter. The bicistronic viral particles also express the Lac Z gene under the control of the a 22 promoter. This will allow us to identify and quantitate the number of infected cells following injection into the rat brain (by staining for Lac Z), and thereby "normalize" behavioral effects to the number of infected target cells. Viral vectors have been generated and qualified for effective gene delivery in (NGF-treated PC12) cells in culture. Viruses are being injected into brain regions (raphe nuclei, locus coeruleus, ventral tegmental area) in adult rats to assess the role of specific gene products in alcohol intake, other behavioral measures and animal models of psychiatric disease.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00016-05 LNG
October 1, 1996 to September 30, 1997

Title of Project: Gene Mapping and Linkage Studies with Short Tandem Repeat (STR) Markers

Principal Investigator: J. Long, Ph.D. (Senior Investigator)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG
R. Kittles, M.S., LNG
E. Moore, LNG
R. Vallejo, Ph.D., LNG

Collaborating Units: NIDDK, NIH (W. Knowler, M.D.; R. Hanson, M.D.; P. Bennett, M.D.)

Staff-Years: 3.5

Sample Type: Human subjects (Interviews) and tissues

Summary of Work: We are searching for genetic linkage between genes that contribute to the predisposition to alcoholism and related behaviors using over 500 highly polymorphic DNA marker loci that span the human genome. To date, we have completed over 200,000 locus typings, primarily on Southwestern American Indians and Finns. We have performed a whole autosomal genome scan for genetic linkage to alcohol dependence in the Southwestern American Indian tribe. Genotypes at 517 autosomal microsatellite loci and clinical evaluations were available for 152 subjects belonging to extended pedigrees and forming 172 sib-pairs. Highly suggestive evidence for linkage emerged for two genomic regions; both regions harbor neurogenetic candidate genes. The best evidence is seen with D11S1984 on chromosome 11p, in close proximity to the DRD_4 dopamine receptor gene. Good evidence is seen with D4S3242 on chromosome 4p, nearby the β1 GABA receptor gene.

In Finns, we have investigated autosomal and Y chromosome DNA variability in relation to the Tridimensional Personality Questionnaire (TPQ) and alcohol dependence and antisocial personality disorder (ASPD). For the autosomal genes, we find evidence for genetic linkage and association between ASPD with alcoholism and the chromosome 6 marker locus D6S286. We also find evidence for linkage and association between ASPD with alcoholism and a polymorphism in the closely linked serotoinin receptor gene HTR_{1B}. With regard to the Y chromosome, we find a significant association with alcohol dependence or abuse and three groups of Y chromosomes that are closely related by their mutational histories.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00017-05 LNG
October 1, 1996 to September 30, 1997

Title of Project: Population Genetics of Native American Tribes

Principal Investigator: J. Long, Ph.D. (Senior Investigator)
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Other Personnel: D. Goldman, M.D., LNG
F. Romero, M.A., M.P.H., LNG

Collaborating Units: Indian Health Service (C. North, M.D.)

Staff-Years: 0.3

Sample Type: Human subjects (Interviews) and tissues

Summary of Work: The purpose of this work is to ascertain the numbers of alleles, allele frequencies, and allele frequency differences among American Indian tribes. The genetic systems being typed are the same as those being used currently in our genetic linkage analyses. Approximately 30 individuals from each of 20 tribes, collected primarily at the Albuquerque Indian Hospital between the years 1992 and 1994, are being analyzed. Various tests for allele frequency differences between tribal groupings based on cultural and linguistic affinities are being performed. This information is important to genetic linkage and disease association studies on Americans Indians because poor estimates of allele frequencies can result in false evidence for linkage and allelic heterogeneity among groups can create spurious associations with disease.

In order to more fully quantify isolate structure and exploit such populations for linkage analyses, we have developed a maximum likelihood method to characterize populations by their levels of gene identity. We have applied this method to microsatellite typings for three American Indian and three European populations. Low gene identity was also observed in Europeans (approximately 28%). By contrast, gene identity was higher in all American Indian populations (39%). We also find that while the overall level of gene identity does not vary much between tribes, there are significant differences in allele frequencies. In comparisons among Southwestern American Indians, we find that genetic affiliations are based on geographic proximity, rather than culture or language groupings. Because of the inter-tribal differences in allele frequencies, it is important that linkage analysis in American Indians proceed with allele frequencies specific for the tribe from which the pedigrees were sampled.

INTRAMURAL RESEARCH PROJECT ZO1 AA 00025-01 LNG
October 1, 1996 to September 30, 1997

Title of Project: Transmission and Genetics of Alcohol Disorders in a Native American Tribe with Low Prevalences

Principal Investigator: J. Long, Ph.D. (Senior Investigator)
LNG, DICBR, NIAAA, NIH
Bethesda MD 20892

Other Personnel: D. Goldman, M.D., LNG
E. Moore, LNG

Collaborating Units: Center for Human Behavior Studies, Weatherford OK (B. Albaugh, MSW)

Staff-Years: 0.33

Sample Type: Human subjects (Interviews) and tissues

Summary of Work: Using a whole genome scan, we will assess genetic linkage to alcoholism and associated psychiatric disorders in Choctaw American Indians. Choctaw is a large Eastern North American Indian tribe with over 30,000 enrolled members living within tribal boundaries in Oklahoma. By contrast to neighboring American Indian tribes that have high prevalences of alcoholism, this tribe stands out because alcoholism has a low prevalence, about 1% and 10% of females and males, respectively. By studying American Indians in the context of low alcoholism, we can expect to reveal different insights into the roles of genetic and/or environmental determinants of alcoholism.

Genetic analysis is to be conducted using three samples from the tribe: a small random sample (N=100), 3 large extended families (N>80 per family), and a sample of more than 150 admixed nuclear families. The admixed nuclear families will be selected to have some Euro-American ancestry and at least one alcoholic family member. The transmission/ disequilibrium test (TDT) is the linkage analysis method of principal interest, because it has been shown to have increased power with population admixture. However, the sampling design will also accommodate standard non-parametric two- and multi-point linkage methods. These methods will be useful for high resolution mapping of promising chromosomal regions identified by the TDT. In order to perform the analyses outlined above, individual psychiatric interviews and blood samples will be collected. Research diagnoses will be made from the psychiatric interviews and DNA for genotyping will be extracted from the blood samples. For the linkage analysis, we expect to type up to 500 short tandem repeat DNA polymorphisms spanning the entire human genome.

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